SPECIFIC APPETITES IN THE DOMESTIC FOWL

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"The necessity of regulating intake of individual food components emerges from a simple fact. For omnivores there is no single natural source of food that contains adequate amounts of all constituents required for optimal living."

Josef Lat.

I declare that this thesis has been composed by me, and that the work described is my own.

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SUMMARY

The development and expression of specific appetites for calcium, sodium and thiamine in the fowl were studied. It was found that calcium-deprived chickens exhibited a preference for diets supplemented with calcium carbonate or calcium lactate. There was no immediate preference, but a group preference characteristically built up over a period of days, suggesting that learning plays a role in the development of this appetite. It is specifically for calcium, in that strontium, the element most closely related to calcium, is not selected. Experiments investigating the importance of visual and gustatory cues showed that selection could still occur when only visual, or only taste cues were available, though selection tended to be less efficient under these circumstances. No appetite could be demonstrated in the complete absence of these cues. A mildly unpleasant flavour such as calcium lactate may act as a helpful cue, but a more aversive taste, such as quinine, blocks the preference for calcium. The importance of hedonic responses is emphasised by the failure of deficient birds to select solutions of calcium salts, though they show a preference for a suspension of calcium carbonate, which is rather more palatable. Calcium deprivation leads to an increase in appetitive pecking behaviour when chickens are presented with a range of different stimuli in an open arena, although deprived and normal birds show similar preferential responses. The effects of deprivation could not be simulated in normal birds by the administration of parathyroid hormone, and it

was concluded that this hormone does not exert a direct effect on the behaviour. An experiment to measure the effects of deprivation on birds in an activity cage yielded ambiguous results. The importance of need reduction in the development of calcium preference was assessed. Exclusive access to a supplemented diet for as long as four days was necessary before a preference was noted. However, injection of a very small quantity of a calcium salt had a profound effect in delaying the development of the preference. Expression of the appetite could not be inhibited by administering drugs - either a depressant, like alcohol, or a true tranquillising agent. Taken as a whole, these results suggest that ingestion of calcium may exert its reinforcing action by means of a general effect on 'well-being', rather than upon some particular site of action, such as the skeletal system, or by means of some specific mechanism, such as anxiety reduction. The fowl differs from the rat in that novelty plays no part in the development of a specific appetite. A less detailed study was carried out on the effects of sodium and of thiamine deficiency. Both normal and sodium-deprived chickens avoid saline solutions. Deprived birds were indifferent to food supplemented with sodium chloride, and there was no avidity for sodium when a functional deficiency was induced by a non-dietary method. The only behavioural effect observed in these sodium-deprived chickens was an increase in appetitive pecking activity, similar to that seen in the calcium-deprived birds. It was concluded that the fowl, unlike the domestic rat and other mammals, does not exhibit a specific appetite for sodium. On the other hand, a specific

appetite for a thiamine-supplemented diet was readily demonstrable after a deficient state had been induced by feeding a metabolic antagonist. The significance of these results was discussed in an evolutionary context, and they were related to the nutritional requirements of the fowl in a natural habitat.

INTRODUCTION

Most research on the mechanisms of specific appetite has been directed to answering one basic question: is a specific appetite developed in the individual organism through a process of learning on the basis of reinforcement, or is there an innate recognition of the appropriate needed substance? Modern ethological theory, of course, rejects this strict dichotomy, but it still remains necessary to assess the importance of unlearnt and of experiential factors in the development of a preference. In the course of investigating these questions, the relative roles of oropharyngeal and postingestional factors in the identification of and preference for particular diets must be assessed. We know almost nothing about how specific appetites have evolved, their phylogenetic distribution, and their presence or absence as a function of food habits and habitat in particular groups of animals (Rozin, 1967).

The vast majority of studies have related exclusively to the domestic rat, and although this work has been fruitful in generating hypotheses, they are of necessity very limited, since the generality of these phenomena has not been established. The study described in this thesis is a comparative one, insofar as an attempt is made to elucidate the mechanisms which underlie specific appetites in the domestic fowl, and then to compare and contrast them with those described in the rat, and other mammalian species.

GENERAL REVIEW OF THE LITERATURE

1. SELF SELECTION IN A VARIETY OF SPECIES INCLUDING FIELD OBSERVATIONS

Under natural conditions the vast majority of animals of a species find and eat an adequate diet. This may be because, in general, natural foods are complex, containing a considerable range of nutrients, and thus even a random choice of foods provides a diet which includes all the components essential to maintenance and growth. It may be, on the other hand, that animals select their food on the basis of physiological needs. It is most likely that both these factors contribute to the balancing of the diet (Anon. 1944).

In a given natural population it will be those animals on the highest plane of nutrition which are most likely to reproduce and rear their young successfully, and so it appears certain that natural selection has exerted a powerful influence upon the ability to choose an adequate diet, by whatever means this is achieved (Dove 1935). There are, however, no direct observations of wild animals in their natural habitat developing nutritional deficiencies similar to those produced in the laboratory, since natural foods are mixtures of essential nutrients, and single components such as those used in laboratory studies are rarely found in the natural environment (Maller 1967). Taking a rather extreme position, Maller (1967) claims that the observations that domesticated animals are able to grow by selecting single food

components, as well as to correct nutritional deficiencies in some cases requiring only microgram quantities, argue for the existence of a similar ability in wild animals.

Work on self selection and the ability of animals to correct specific deficiencies could only be pursued on a scientific basis after the nutritional studies of the early years of this century had begun to identify in detail components such as vitamins and minerals, which in addition to protein, carbohydrate and fat, were necessary to provide a complete and adequate diet for maintenance, production and growth.

The earliest experimental work on the ability of animals to select their own food appears to be the finding of Evvard (1915) that pigs under a free choice system of feeding could select for themselves a balanced diet that allowed excellent growth. He believed that the pig's appetite was a reliable indication of its physiological needs; protein intake in particular conforming very closely to the amount of flesh the animal was laying down. In essence this was confirmed by Braude (1948) who reported that pigs fed on the self choice system gave larger daily gains in body weight than those fed a mixture in the normal way. However, his numbers were small and he concluded that the pigs showed considerable individual taste in their preferences.

Some of the earliest experiments on self selection relate to chickens. Pearl and Fairchild (1921) found that when allowed

to select their own diet from natural food components, chickens consumed 33·4% less protein and 33·7% less fat and had a total calorie intake 8·4% lower than the control birds who were fed the regular Maine Station ration. Their egg production was excellent and both groups showed similar gains in body weight. The authors concluded that when fowls were allowed to choose the kind and amount of their food they made a better physiological utilisation of their ration than did birds under mass feeding.

On the other hand Dove (1935) described how some chickens (in small groups) selected feed ingredients that supported a high growth rate, while others chose foods that promoted a slow rate. He then took groups of test chickens, and to some he fed the mixture which had been selected by the high growth rate birds, while to others he gave the diet chosen by the birds which had shown the slow rate of growth. The first group grew faster than the second, indicating that the ration they were receiving was nutritionally superior, but not as rapidly as the original group which had selected the identical diet for themselves, which suggested that the self selection group were making a better physiological utilisation of the diet. Dove (1935) emphasises the importance of social factors in this situation, and postulates that 'leaders' can impress their own food preferences on the group as a whole. It would appear from this work that not all chickens are endowed with a high degree of nutritional wisdom, since within the experimental group Dove demonstrated this wide

spectrum of ability in self selection.

Funk (1932) offered White Leghorn chicks 9 common components of a diet for 10 weeks and found that selection was variable the first two weeks but quite uniform thereafter. He noted that their growth was normal, although slightly below that of the controls fed a standard ration, and also that they were more variable in their conformation. Graham (1932) presented a very wide choice of feedstuffs to chicks from which they selected their own diet, and he stated that texture had a considerable effect upon the palatability of foods. The most noteworthy features of his experiment were the large quantities of wheat germ and the very small amounts of minerals consumed, and there was an interesting seasonal variation in cod liver oil intake - which was fifty times higher in winter than in summer. This finding Graham ascribed to the increased requirement for Vitamin D in winter, but it also seems possible that it could have been related to an increase in rancidity, and thus unpalatability, in summer.

Stearns and Hollander (1939) reported that pigeons were able to select their diet successfully from a cafeteria type of feeder, similar to that used by Graham.

Pullets were offered whole grains and a balanced mash supplement by Graham (1934) who found that intake varied considerably from one bird to another and within any individual from day to day, yet in general the level of protein intake remained fairly

constant and most birds laid and grew well.

In a brief communication by Banta (1932) the author described how individually housed laying hens were allowed to choose their diet from 13 different foodstuffs (4 cereals, 4 protein sources, 2 brans, calcium grit, insoluble grit and salt) for a period exceeding six months. In general these birds made a poor selection, on average consuming too much cereal, and considerably too little protein, calcium and salt.

These findings are not easy to interpret, but all the authors cited, with the exception of Banta (1932) and Graham (1934), were recording the food consumed by either small or large groups of animals. The two latter authors, recording the intakes of isolated individuals, reported an apparently inferior performance by their experimental animals. It may be possible to infer therefore that a number of animals feeding together as a group may make a more beneficial selection than they would if feeding as separate individuals. Indeed, this effect has been investigated in rats by McDonald, Stern and Hahn (1963), who offered rats a selection of dietary components in different containers. They reported that in groups all rats showed good weight gains, while about 50% of the individually housed animals initially failed to ingest sufficient of the protein source (casein), and thus lost weight. They accounted for this finding in terms of "optimal environmental stimulation", considering that factors other than social facilitation were partly responsible,

but that these factors at present were unknown.

However, there may be a simpler explanation. If, as described by Dove (1935) each individual exhibits a slightly different spectrum of food preference, it will be those animals which make the most appropriate choice which will be the strongest, grow the fastest, and tend to emerge as dominant in the group. Turner (1965) has shown that a bird is more likely to consume an unfamiliar food if it is given the opportunity to observe another individual eating it. This form of social facilitation allows one to postulate a mechanism whereby the fastest growing birds, which will probably also be the birds which spend most time eating, may influence the feeding behaviour of an entire group in a beneficial direction. This could explain why a social group apparently makes a more appropriate choice under free selection conditions than separate individuals, and shows that this superior performance may be in some sense an artifact.

A comparison of the findings of Banta (1932) and Graham (1934) suggests that chickens may be capable of selecting an appropriate diet when they have to supplement a cereal base with a balanced protein concentrate, but that making an appropriate choice from a number of cereals, and a number of protein foods, some of which may be rather unpalatable, is a task they do not perform well.

Comparative studies of self selection abilities have been carried out in a number of species.

Davis (1928) working with human infants, reported that 1 to 2 year old children selected an adequate diet from a wide range of foods. In general they preferred fruits, milk, whole grain cereals, meat and eggs, but the experiments were poorly designed in that the range of complete foods provided was so broad that an entirely random choice would probably have been equally well balanced.

There is one report that animals may show selection in favour of iron. Jones (1918) found that sheep, in an area of Australia where the herbage was deficient in iron, could be seen eating earth at points where outcrops of ironstone occurred. He suggested that this was in response to an iron deficiency anaemia produced by parasitism with nematodes.

Godden (1926) stated that sheep grazing both hill and cultivated pastures deficient in minerals, tended to select plants with a higher ash content, leaving the deficient pasture untouched, although in calorific value it was not inferior to that eaten. Similar findings were reported by Weir and Torrel (1959) who found that when rumen contents obtained through a ruminal fistula were compared with herbage obtained by random harvesting, the sheep were consistently selecting forage higher in protein and lower in crude fibre. Miller (1968) has shown that grouse, hares and rabbits selectively graze small (1 m^2) patches of heather which have been fertilised in preference to those which have been not. Grouse showed a preference for nitrogen in winter only, and no preference

for phosphate, while hares and rabbits exhibited a preference for heather rich in both nitrogen and phosphate in winter, and for nitrogen only in summer. The author ascribes the seasonal difference in selection to the fact that both these species live almost exclusively on heather during the winter, so that it has to provide all their nutritional requirements during this period, whereas in summer they can balance their diet by feeding on several different kinds of plant.

An appetite for phosphorus was first described by Green (1925), who found that South African cattle, from areas where the pasture was low in phosphorus, had developed the habit of eating bones. However, in severe cases, they would also chew tin cans and pieces of wood, so it seemed most likely that it was a generalised hunger rather than a specific appetite for phosphorus.

Gordon, Tribe and Graham (1954) agreed with this conclusion. They provided calcium supplements, with and without phosphate, to cattle and sheep in an area of Skye where the soil was phosphorus deficient. Although, over a period of several months, the animals consumed a little more of the phosphate-rich supplement, the difference was not significant and their growth remained poor. These authors concluded that the osteophagia seen under these conditions primarily serves to increase the calcium intake, and that the generally depraved appetite seen when animals are severely phosphorus deficient is not accurately directed towards phosphate sources.

However, under certain circumstances, an appetite for phosphorus does seem to be present. Stoddard and Mickelson (1961) found that cows given food mixes without added phosphate would consume considerably more of a phosphate supplement, available ad libitum, than would controls. They stated that palatability was important, for the size of the intake was very dependent on the nature of the supplement - dicalcium phosphate being more acceptable than bone flour.

Nevens (1919) recorded the food intakes of individual dairy calves offered a free choice of hay and a balanced supplement in addition to milk, from the age of 3 days until they were six months old. He noted that they ate more food and gained more weight than controls fed the fixed, recommended quantity in two daily feeds. Satisfactory results were reported by McCandlish (1923a, 1923b, 1924) too in rearing young calves by means of the self feeder from the age of 16 days upwards. Calves were reared in small groups of three or four, and were offered a free choice of various dietary components for periods of up to 27 months. Their growth was faster than that of controls and they appeared to balance their ration well with respect to calorific value, protein and salt intake.

It would therefore appear from this work that, apart from a tendency to lay down excessive amounts of fat, young cattle are able to adjust satisfactorily to a self selection regime. However, Tribe (1950) is sceptical of the ability of

the ruminant to balance its diet, and reports that sheep fed on a self choice system which includes linseed cake meal select a diet with approximately twice as much protein as is theoretically necessary. If the linseed is replaced by white fish meal, less protein than is required is consumed. Clearly, the palatability of the protein source is taking precedence over the physiological requirements.

Pregnant sheep are notoriously poor at balancing their diet. Gordon and Tribe (1951) offered eight pregnant Cheviot ewes a free choice of protein and carbohydrate sources, roughage, minerals and salt from conception to lambing. The ewes were individually housed indoors, and ate too much early in pregnancy and too little as lambing approached, and in addition their protein intake was too low. The initial excessive intake meant that they became overfat, and so they were reluctant to rise to their feet in order to eat, during the final stages of pregnancy when their intake should ideally have been maximal. This low and decreasing plane of nutrition resulted in both heavy ewe and lamb mortality.

The importance of palatability is emphasised by another experiment. When sheep were given a choice between maize, milo or wheat which had been prepared in different ways, being either whole, finely ground, crimped or pelleted, there was a preference for preparation, but not for different grains (Brown and Caveness 1959). Blindfolding the animals and interfering with their sense

of smell affected preference very considerably. The authors concluded that sheep clearly select their feed by appearance and odour, rather than by content.

2. SELF SELECTION OF DIET IN THE LABORATORY RAT

In every case of self selection reported in the previous section (excepting McDonald, Stern and Hahn 1963) the dietary components offered for selection consisted, in the main, of natural foodstuffs, and thus, to a greater or lesser extent, comprised a mixture of protein, carbohydrate, fat, minerals and vitamins. In experiments where large animals are used it is not feasible to work with purified or synthetic foodstuffs because of the prohibitive cost. The advantage of the laboratory rat in nutritional studies is that only small quantities are required, and so the use of pure dietary components becomes a practical proposition. It is for this reason that so much of the work on self selection and on specific appetites is based on the rat, but it is also most unfortunate, since clearly there are great comparative differences between species in this field.

Early experiments, offering a small number of choices, suggested that rats were able to select a suitable diet. Osborne and Mendel (1918) found that, in general, rats preferred foods with an adequate protein or vitamin content to those which were deficient, and grew satisfactorily. The number of animals they used was rather small, though, and no firm conclusions were drawn.

It was also shown (Mitchell and Mendel 1921) that both rats and mice (with a few exceptions) exhibited a preference for a high protein casein food, rather than a low protein, zein diet, and also that they preferred foods high in Vitamin A, Vitamin B and salt to foods deficient in these components. Most of the animals selected the adequate diets and their growth was comparable to that of the controls.

Other findings, however, suggested that rats were unable to choose a suitable diet under all circumstances. Beadles, Braman and Mitchell (1930) stated that rats could not distinguish (as determined by the quantity consumed in paired feeding) between a diet deficient in an amino acid (cystine), and the same diet with added cystine. Kon (1931) gave his experimental animals a definite amount of yeast extract, and then offered sucrose or rice starch, caseinogen and a salt mixture in separate feeders to these young rats. Confronted by these separate dietary components in purified form, the rats on average chose a diet containing only 6·5% of protein, and showed weight gains greatly inferior to controls fed a stock diet of the same ingredients providing 20% of protein. In view of other work to be described later, it appears possible that a crucial variable in this experiment may be the age of the rats.

Harlow (1932) was interested in identifying the cues used by rats in selecting foods. He fed normal rats twice daily, allowing them to choose any of four foods presented simultaneously. They selected a standard balanced ration or meat scraps equally

often and preferred these to cheese, which in turn was preferred to corn, oats and sunflower oil. Rats made blind, anosmic, or both, showed the same preferences, which indicated that the food choice was probably based on gustatory or tactual cues. Tribe and Gordon (1955a) deprived rats of their olfactory bulbs, deprived them of the Vitamin B complex by feeding a deficient diet, and then offered them a choice between two foods, identical except in that one contained adequate amounts of the Vitamin B complex while the other was deficient in it. These rats showed a preference for the enriched diet, and since their performance closely paralleled that of normal rats, the authors concluded that the sense of smell is not of critical importance in the selection of diet by rats.

As many as 10 different purified food components in solid and liquid food were presented to rats by Richter, Holt and Barelare (1938) who demonstrated that from feeders containing casein, sucrose, yeast, olive oil, cod liver oil and a variety of mineral salt solutions, the animals could select amounts of food such that their normal growth and reproduction were maintained. Their daily caloric intake was 18% below that of controls, suggesting that physiological utilisation was superior. From these results the authors concluded that the rat has special appetites for carbohydrate (sucrose), protein (casein), fat (olive oil), sodium (as chloride), calcium (as lactate), phosphorus (sodium phosphate), potassium (as chloride), Vitamins A and D (as

cod liver oil), the Vitamin B complex (yeast) and Vitamin E (wheat germ oil). This conclusion would appear to be somewhat extreme, based as it is on no more evidence than that the rats consumed some of all these components. Richter and Barelare (1938), extending their study to pregnant and lactating rats, found that during pregnancy the appetites for casein, olive oil, sodium chloride, sodium phosphate and calcium lactate all rose appreciably, that for yeast rose slightly, and that for sucrose decreased. During lactation the intake of casein and olive oil rose still further. They stated that the ability of animals to make dietary adjustments which help to maintain physiological equilibrium was demonstrated, - even during such altered states as pregnancy and lactation.

On the other hand, a dissenting note is provided by later workers. Scott, Smith and Verney (1948) reported that when female rats selecting their own diet (from sucrose, fat, casein and salts, - vitamins being force fed) became pregnant, there was an increase in total intake but little change in the proportion of each component consumed. That the diet selected was in some respect inadequate was suggested by the fact that they invariably ate their young within a few days of parturition. This finding is supported by an experiment carried out by Tribe (1955). Rats selecting their diet from a total of eight purified dietary components during pregnancy chose a diet deficient in calories and protein, and including a lower level of salt and mineral

mixture than they had consumed prior to pregnancy. Again, heavy cannibalism of newborn pups was reported.

Most workers, however, do appear to agree that, in general, adult rats are capable of an appropriate choice under self selection conditions. When rats were permitted to select their own diet from cups containing four pure dietary components (sucrose, fat, casein, salts) the pattern was subject to considerable individual variation. Although most of the rats ate fairly uniform quantities of sucrose and fat, some apparently found casein palatable, ate over 3 grams per day and thrived, while others ate less than 0·1 g. daily, and died (Scott 1946). A similar individual variation is reported by Tribe (1955). Thirteen out of fifteen adult females made a satisfactory adjustment to a self selection regime involving 8 different components, but the remaining two both died. Pilgrim and Patton (1947), offering the same selection as Scott (1946), found that about one third of their rats grew at a subnormal rate owing to an inadequate consumption of casein, and concluded that though the appetites for calories and salt were related to need, that for protein was not.

This ability of the rat to regulate its calorie intake was emphasised by the finding of Hausmann (1932), that when rats are given alcohol or sugar solution to drink in place of water, they reduce their food intake by an amount corresponding to the additional calorie supplement they are receiving from their liquid intake. There is no reduction if they receive saccharin in place

of sugar solution (Hausmann 1933), suggesting that this control is dependent upon central mechanisms rather than upon peripheral signals.

There is some evidence that an ability to adapt successfully to a self selection regime may depend upon the age at which the rat is first exposed to it. Young weanling rats show a marked inability to select a balanced diet, eating less casein and fat and more sucrose than adults. (Scott, Smith and Verney 1948). Tribe (1955) too reported that almost all young rats introduced to the routine for the first time at the age of 21 days, immediately after weaning, failed to make an appropriate selection, and died. It is not established whether the factors important in this situation are maturational or experiential.

The importance of the nature and palatability of the source of protein was demonstrated by Scott and Quint (1946) who allowed rats to make their choice from diets containing casein, lactalbumin, fibrin or egg albumen, and found that most animals would eat casein, lactalbumin or fibrin, whereas very few found egg albumen acceptable. They concluded that an appetite for protein is not a true specific appetite, being based on a simple preference, although it can be a learned appetite.

3. SODIUM APPETITE

The existence of an appetite for salt in herbivorous animals has long been a common observation (Anon. 1944). In Africa, wild game are attracted to salt licks, and this occurs

also with kangaroo in the mountains and plains of Australia. Rocky Mountain elk show salt hunger in early summer after feeding on succulent forage for two to three weeks, and are attracted both to natural licks (either saline springs or soil rich in sodium) and to salt blocks. Sodium deficient sheep with parotid fistulas select plants with a higher sodium content than do control animals (Denton 1967). Rabbits in an area of the Snowy Mountains where the soil is sodium deficient will gnaw at wooden pegs impregnated with sodium chloride or sodium bicarbonate, while in general ignoring those which contain potassium chloride or magnesium chloride (Blair-West et al. 1968).

This appetite for sodium is probably the most documented and the best understood of all the specific hungers. Richter (1936) found that when rats were adrenalectomised - an operation which increases loss of salt in the urine - and then offered a choice between tap-water and sodium chloride solution, they chose the salt at concentrations of both 1% and 3% - the latter a level that normal rats find highly aversive. If no salt solution was available their mortality was much higher. Later work (Richter and Eckert 1938) showed that adrenalectomised rats exhibited this preference for sodium lactate and sodium phosphate also, but not for sodium iodide (which is toxic), and that in single choice experiments there was no increased appetite for the chlorides of iron, potassium, magnesium, calcium, aluminium or ammonium. However, in a multiple choice experiment there was a small increase

in appetite for potassium chloride.

The existence of this preference for salt by sodium-depleted rats was confirmed by Mark (1942) who stated that the increased appetite for salt fell back to normal following the implantation of deoxycorticosterone acetate. Clark and Clausen (1943) adrenalectomised rats which were being fed on a self selection regime. The only change in feeding behaviour observed was an increase in appetite for salt, which could be reversed by adrenal cortical therapy. The adrenalectomy had no effect upon the intake of protein, carbohydrate, fat, yeast or mineral mixture.

A slightly dissenting note was introduced by Bare (1949), who found that normal and adrenalectomised rats behaved similarly in that both showed a preference for sodium chloride solutions at concentrations below 0·9% and an aversion for it at stronger concentrations. However, the adrenalectomised rats ingested more NaCl than the normal rats at all concentrations. Young and Chaplin (1949) reported rather similar results in that their rats, both normal and adrenalectomised, also exhibited this spectrum of preference with maximal intake at an optimal concentration of 0·7% (0·9% was not tested). Their subjects' daily intake of sodium chloride was dependent only upon the concentration offered, and they concluded that there is no homeostatic regulation of the intake of sodium chloride as such. In addition their adrenalectomised rats did not show any increase in sodium intake over the controls, and they attributed this to the fact that even the

controls were ingesting daily an amount vastly in excess of their needs. There does appear to be some doubt though as to whether their subjects had been completely adrenalectomised, so perhaps too much significance should not be attached to these findings.

Numerous workers have investigated the specificity of this sodium appetite. Fregly (1958) depleted rats of sodium by means of adrenalectomy, and investigated their response to a number of salts. He found generalistion to sodium sulphate, sodium bicarbonate and sodium citrate, but not to sodium oxalate or sodium iodide. Potassium, calcium and lithium chlorides were also rejected. The rats showed weight gains only when sodium chloride was available, suggesting that only in the case of this salt did intake match requirements. Bell and Williams (1960) found that calves depleted of sodium by external canalisation of the parotid duct showed a preference for both sodium chloride and sodium bicarbonate. Beilharz, Denton and Sabine (1962) reported a similar finding in sheep. Falk (1965) depleted rats of sodium by intraperitoneal dialysis, and found that there was no difference in preference between potassium and sodium chloride solutions of equal acceptability, when they were presented simultaneously, and also that potassium chloride was preferred to tap-water. In short term preference tests Nachman (1962) demonstrated a preference in adrenalectomised rats for sodium chloride, nitrate, sulphate, iodide, and acetate. There is also an immediate

preference for lithium chloride (which is toxic), but after 24 hours this has become an aversion (Nachman 1963a). These experiments suggest that the immediate, or short term, sodium appetite may generalise to a number of sodium salts, and also to other salts such as potassium chloride and lithium chloride. After a certain period, however, the appetite appears to become much more specific, and in the case of salts which are toxic, such as sodium oxalate or iodide, or lithium chloride, the initial preference has been altered to an aversion, suggesting that feedback factors may play an important part in the maintenance of the preference.

In all the preceding experiments the sodium has been presented in the form of a solution, but when sodium chloride is made available in the food instead, there is apparently no preference for it as opposed to a deficient food, in a two choice test. At low levels of 0·1% - 1·0% sodium chloride, both sodium deprived rats and normal controls show a preference for the enriched diet, while at higher levels of up to 2·5%, both groups showed an aversion for it (Scott, Verney and Morissey 1950a). Bolles et al. (1964), on the other hand, presented normal and adrenalectomised rats with either a wet mash containing 5% sodium chloride, or the same food without added salt, after a 24 hour period of food deprivation. Whereas the controls showed a strong aversion to the salty mash, the sodium deprived animals ate it avidly. In Scott, Verney and Morissey's experiment, although no

preference for sodium chloride, phosphate or sulphate was observed, there was a significant preference for sodium bicarbonate. The methodology of this experiment also differed from earlier ones in that the rats were depleted of sodium by feeding a diet deficient in sodium, rather than by adrenalectomy or intraperitoneal dialysis. However, Nachman and Pfaffmann (1963) also deprived rats of sodium by the feeding of a deficient diet, and found that the depleted animals ingested significantly more sodium chloride solution (at concentrations of both 0·6% and 2·4%) than did normal subjects. These two results suggest that, in the rat, sodium depletion can be produced by feeding a low salt diet, and that sodium depleted rats will select foods high in salt to which normal rats show an aversion, so that the significance of Scott et al's (1950) results must remain something of an unknown quantity.

4. MECHANISMS UNDERLYING THE PREFERENCE FOR SODIUM

Two problems are involved in the understanding of the regulation of sodium intake. The first concerns the nature of the cue which makes homeostasis possible, and the second is the mechanism of the regulation itself, whether for example it involves feedback, and if so, of what kind.

McCance (1936) carried out a study in humans which provided some interesting subjective evidence. Men were made salt deficient by sweating, while on a low sodium diet, and they

showed a dulling of mental processes which was associated with anorexia, nausea, muscle cramps and fatigue. A peculiar sensation developed in the mouth and all food seemed tasteless. An experiment of washing out the mouth with salt and water appeared to restore the sense of flavour, and was considered very refreshing. One subject recognised the symptoms as thirst, while another regarded it as quite different from thirst, and one subject longed for salt while another had no special craving for it.

The necessary antecedent of behavioural adjustment is some sort of alteration of the internal environment (Epstein and Stellar, 1955) and it had long been assumed that plasma sodium was the cue monitored, but recent work has questioned this assumption (Smith, Holman and Fortune, 1968). Low levels of plasma sodium can be produced by hydration, and this does not elicit an increased appetite for sodium (Stricker and Wolf, 1966). The converse is also true, for Beilharz and Kay (1963) depleted sheep of sodium by means of a parotid fistula, and found that intravenous injections of sodium chloride did not reduce the amount of sodium bicarbonate solution drunk immediately afterwards, even when plasma sodium levels were raised well above normal. They found that the appetite for sodium could be slightly reduced by giving 1·5 litres of water into the rumen 10 minutes before the sodium drinking period, but that no further reduction occurred when a solution of sodium bicarbonate was given instead, and concluded that the appetite for sodium is not dependent upon the

concentration of sodium in either the plasma or the rumen contents. In rats, too, stomach loading of sodium chloride is ineffective in abolishing sodium appetite, whether the rats have been depleted by adrenalectomy (Nachman and Valentino, 1966) or by dialysis (Falk and Lipton, 1967). The rats were depleted of sodium by dialysing isotonic glucose solution into the peritoneal cavity and removing it again. A sodium depletion effective for only 3 hours and followed by massive repletion, the sodium chloride solution being given directly into the bloodstream, is sufficient to initiate and maintain a long-lasting appetite for sodium. This remarkable finding suggests that a comparatively brief period of sodium depletion 'switches on' some central mechanism governing the appetite for sodium, and that a form of sodium seeking behaviour persists until 'switched off' by peripheral signals indicating the presence of sodium. This sequence of events bears a striking similarity to the 'psychohydraulic' model of behaviour postulated by Lorenz (1950), in which action specific energy builds up and is then conserved until the stimulus situation is appropriate to its release.

One of the major adjustments to sodium depletion is an increased rate of aldosterone secretion (Blair-West et al., 1963). This cannot be the cue monitored either, as it rapidly reverses with sodium repletion, and so cannot function as an enduring deficit stimulus (Falk and Lipton, 1967), while of course sodium appetite also develops in the adrenalectomised animal in the

absence of aldosterone (Richter, 1936).

It would appear that blood volume is an important factor, in view of the finding (Wolf and Stricker, 1967) that hypovolaemia alone, without any changes in plasma sodium levels, can elicit a sodium appetite in both normal and adrenalectomised rats. Nevertheless, it can only be concluded that final identification of the cues has not yet been made.

Secondly, there is the problem of how the cue is utilised. Two main theories have been postulated. The first is the avidity theory, which implies that the nervous system is somehow directly affected by low sodium levels in such a way that the taste of saline is preferred, (Katz, 1937; Smith, Holman and Fortune, 1968) or even that the threshold for the discrimination of sodium chloride becomes more sensitive (Richter, 1939).

Richter (1939) discovered that the sodium preference threshold for normal rats was 0·055%, while for adrenalectomised rats it was 0·003%, and he found that the adrenalectomised rat showed a preference for these extremely small amounts of salt even when the concentration was increased upwards from subliminal levels. This indicated that the preference was exhibited before the rat had had the opportunity of experiencing the beneficial effects of ingesting large amounts. He concluded that the increased salt intake must have depended on an altered appetite for salt, rather than on a trial and error process resulting in relief from insufficiency symptoms after ingesting salt, and he ascribed this to an increase in the sensitivity of the receptors. However,

Pfaffmann and Bare (1950) exposed the chorda tympani of the rat and made direct recordings from the nerve fibres, while stimulating the tip of the rat's tongue with either salt solution or tap-water. They found that the threshold for normal rats was 0·008% and that for adrenalectomised rats was 0·010%, and they concluded that in normal rats the sensory threshold is considerably lower than the preference threshold, while in adrenalectomised animals the sensory and the preference thresholds nearly coincide, indicating that a preference test is not per se a measure of peripheral sensitivity. This result was confirmed by Carr (1952) who found the threshold to be 0·009% before adrenalectomy, and exactly the same afterwards. He used a test in which rats were conditioned to drink a very weak salt solution after water deprivation. They were offered both water and saline in a two choice situation, and were given an electric shock if they were still drinking the water after 10 seconds. In this situation, where the normal rats as well as the adrenalectomised animals were highly motivated to select saline, there was no difference between the groups.

Epstein and Stellar (1955) deprived adrenalectomised rats post-operatively of salt for several days, and then compared their intake of sodium chloride with that of other adrenalectomised rats which had had access to it throughout. They found the immediate intake to be at a high level, and comparable with that of the rats whose preference had built up gradually to high levels

over a period of days. This finding supports Richter's conclusion (Richter, 1939) that learning is not of prime importance in this preference - the salt level of the internal environment being the most important factor.

Bare (1949) came to the conclusion that excitation of the taste buds by sodium ions actually evokes salt ingestion, and that as excitation increases to a given amount the intake of salt solution increases, while beyond this point (which closely corresponds to the level of extracellular sodium chloride) further excitation results in a decrease in salt ingestion. Bare suggests that perhaps the normal animal is protected against specific food deprivation by this unlearned stimulus-response connection, and that whenever sodium chloride is available in the environment, it is ingested, the amount of ingestion depending on the strength of the gustatory stimulus.

Some support is lent to this theory by experiments on taste denervation. When all the nerves supplying the tongue - namely the chorda tympani, the lingual nerve, the glossopharyngeal, and the pharyngeal branch of the vagus - are cut in rats, salt intake did not increase following adrenalectomy as it would normally have done, and all the sodium depleted rats died (Richter, 1956).

On the other hand, sheep with a parotid fistula which become severely salt depleted, and whose taste buds have been denervated in a similar fashion, have no difficulty in selecting

sodium bicarbonate solutions in preference to water, and they maintain themselves in good health. They appeared to distinguish the two fluids by smell (Beilharz and Kay, 1963), whereas in rats removal of the olfactory lobes in no way impaired their performance in selecting sodium solutions (Richter, 1956).

These findings suggest, of course, that there may be important and fundamental differences in the development and expression of sodium appetite between different species - it would certainly appear that in the sheep gustatory feedback may be of lesser importance (unlike the rat) and that in this species some additional mechanism may be operating.

This now leads on to the second theory, which states that the preference is not immediately present in its full strength, but that some kind of learning process is involved before the preference can be fully expressed. Some suggestion that this might be so was provided by the early finding that there was a degree of latency (in some cases measured in days) before the preference for sodium became apparent (Richter and Eckert, 1938). However, the main proponents of this theory were Smith, Pool and Weinberg (1958) who depleted rats of salt by the infusion and removal of glucose solution intraperitoneally, and then fistulated the oesophagus in half the animals so that drinking was sham. One group was now given water to drink and one group saline. It was shown that:

1. Both sham and real drinking groups displayed a preference for

saline, whether a deficiency existed or not.

2. Sodium depleted animals showed a greater fluid consumption than controls (which had received isotonic salt solution intraperitoneally).

3. Only the real drinking group showed an enhanced acceptance of saline by salt deficient rats - in the case of sham drinking there was no evidence of a need related preference. This finding was taken as evidence for a learning interpretation of salt craving rather than the avidity theory, but it is not a convincing conclusion in view of several other demonstrations that there is a very rapid development of a need related sodium preference. For example, Falk and Herman (1961) found that rats depleted of sodium by dialysis showed a preference for 3% sodium chloride (which is normally aversive) over distilled water, and that this preference was exhibited over periods as brief as 15 seconds and amounts as small as 1 ml. Since under these conditions there is no opportunity for sodium repletion, the indication is that the selection of sodium is not dependent upon post-ingestational reinforcement. Similar results were reported by Nachman (1962) after exposures as brief as 5 seconds, and by others (Bolles, Sulzbacher and Arant, 1964; Handal, 1965).

Smith, Holman and Fortune (1968) suggest that both the innate and the learning mechanisms operate simultaneously, but that the taste stimulation factor is much the more important. They used the technique of crossed intragastric injections whereby

solution A is ingested by mouth while solution B is injected directly into the stomach, while when solution B is orally ingested, solution A is injected intragastrically. These workers found that whereas normal rats preferred glucose solution to saline, sodium depleted animals preferred saline, and that this was so even when crossed intragastric injections equated stomach contents, so that all animals ingested the same amount of sodium. This clearly suggests that a certain volume of saline ingested orally is more reinforcing than the same amount given directly into the stomach. However, when intragastric injections of glucose or of saline were linked with the presentation of two unpalatable solutions, in the case of depleted rats that solution which was paired with the saline was preferred. This seems to indicate that feedback does make some contribution to a preferential reaction, and is supported by the finding that the preference for saline is much more marked when it is paired with intragastric saline rather than with glucose.

Some additional support is provided by Thomson and Porter (1953) who found that sodium deprived, ageusic and anosmic rats were unable to learn to run a T maze for sub-threshold saline solution using normal training procedures, but that learning occurred when they were forced to make the correct choice eight times in succession over a period of 2 days. The authors concluded that need reduction can serve as reinforcement independently of direct sensory reward, and this suggests that learning may be of

importance in situations where an immediate sensory preference cannot be expressed.

The need for sodium is not always directly associated with its intake, and Harriman (1955) has demonstrated that under some circumstances, preference can take precedence over need. Normal rats were given access to 8% sucrose solution, while a second group was offered distilled water only. Half of each group was now adrenalectomised while the controls were sham operated, and all groups were given access to sugar and salt solutions. The adrenalectomised rats with a pre-operative preference for sugar ingested significantly less salt than those without such a preference, and indeed no more salt than either of the sham-operated groups. This led to heavy mortality in this group, although by the end of the 15 day period of observation the sugar preference was being over-ridden in the survivors, and salt intake was rising. It is clear that under more normal circumstances, however, the intake of sodium chloride solution of certain concentrations is in itself rewarding, quite apart from the preference for it exhibited by normal rats (Bare, 1949; Young and Chaplin, 1949).

Lewis (1960) adrenalectomised rats and trained them to lever press for 1% NaCl. These sodium depleted rats quickly learnt this task, whereas normal controls would not respond, even though they consume 1% NaCl in preference to water. The sodium depleted animals learnt to run a T maze for 1% NaCl as quickly as

normal rats learnt to run the maze for food when hungry, suggesting that they were highly motivated. In addition, they would discriminate between sodium and food deprivation when running the maze.

This approach was extended by Quartermain, Miller and Wolf (1967), who depleted rats of body sodium by the subcutaneous injection of formalin solution, and found that the rate of responding increased with rising dosages of formalin, indicating that previous experience is unnecessary for adjustment of performance to varying levels of need. Similarly, when rats were chronically depleted by either adrenalectomy or by treatment with DOCA, and allowed to lever press either immediately or after 24 hours of deprivation, the rate of responding was greater at 24 hours, in the very first session, and this initial difference was not enhanced by further experience.

Krieckhaus and Wolf (1968) trained thirsty rats to press a lever for either distilled water or 0·9% NaCl, and then tested them in the same situation except that they were now neither thirsty nor rewarded - that is, it was an extinction procedure. Rats which had received the sodium salt when they were thirsty, and were depleted of sodium (by means of subcutaneous formalin) before testing, pressed the lever 2-3 times more than the controls. These controls comprised sodium depleted rats trained with water, calcium chloride or potassium chloride, rats trained with water or NaCl but not depleted prior to testing, and rats trained with

sodium chloride and sodium depleted, but repleted with sodium prior to testing. This experiment is a remarkable demonstration of latent learning, for when the stimulus was first presented no need existed, or indeed had ever existed in the animal's lifetime, and later when a state of need was induced, the animal responded appropriately, even though none of the responses was reinforced. It is also a clear indication that sodium appetite is dependent upon an innate mechanism, for at no time did the rat have an opportunity to associate the taste of sodium chloride with the experience of beneficial post-ingestational feedback.

The ability to learn to make an instrumental response in order to obtain sodium is not confined to the rat. Baldwin (1969) was successful in training goats to press a panel to receive sodium bicarbonate when they had been depleted of sodium by means of a parotid fistula. When given access to salt licks, or to sodium bicarbonate 1% solution ad libitum, they no longer responded. The response rate was not affected by intra-carotid injections of NaCl immediately before testing, even though they raised intra-cephalic sodium levels well above normal. This, again, is evidence against the presence of a monitoring centre for plasma sodium situated in the brain.

In an attempt to locate anatomically the site of the mechanism which controls sodium appetite, Novakova and Cort (1966) produced lesions in the ventromedial nucleus of the hypothalamus of rats. These rats (and their controls) were then depleted of

sodium by the feeding of a low sodium diet and the administration of hydrochlorothiazide. They were then offered tap-water, physiological saline and 20% NaCl solution. The normal rats increased their intake of salt, and returned to sodium balance within 3 days, but the lesioned rats failed to show any compensatory increase. Rats with lesions of equivalent size at any other site behaved as normal animals. The authors conclude that the mechanism which governs the response of the rat to sodium deficit must be located in the ventromedial nucleus of the hypothalamus, but add that it is not clear whether its action involves direct perception of an afferent signal, or the processing of a signal pattern reflected from another central nervous site. That it is more likely to be the former is suggested by the fact that, unlike controls, these animals with lesions in the ventromedial nucleus fail to reduce their food intake following an oral or subcutaneous salt load. This finding, too, suggests that both food intake and salt intake are controlled by either the same centre, or by closely associated centres, in the ventral hypothalamus. The food intake of these rats was not recorded, but it seems doubtful that the failure to increase salt intake could be governed by a disturbed food intake, since the rats had to consume all their food in a 2 hour period per 24 hours.

5. CALCIUM APPETITE

Richter and Eckert (1937) demonstrated that after parathyroidectomy rats exhibit an increased appetite for calcium.

Daily consumption of 2·4% calcium lactate solution increased on average by a factor of four in 17 out of 18 rats, following removal of the parathyroid glands. If surgical transplants of parathyroid tissue were now made into the eyeball (to avoid rejection dangers) the calcium appetite returned to normal. The authors noted that these parathyroidectomised rats showed a concomitant aversion to sodium phosphate, which is interesting, since the ingestion of phosphate salts would exacerbate any existing deficiency of calcium.

In a later paper (1939) these workers found that the calcium appetite of such parathyroidectomised rats generalised to other calcium salts and even to other elements. Increased appetites were seen for calcium lactate 2·4% (26 out of 27 animals), calcium acetate 0·1% (3 out of 3 animals), calcium gluconate 0·3% (3 out of 4 animals), and calcium nitrate 0·5% (3 out of 3 animals). Appetite was reduced for sodium phosphate 4% (5 out of 6 animals), but increased for strontium chloride 0·02% (3 out of 4), strontium lactate 0·02% (2 out of 3) and for magnesium chloride 0·5% (3 out of 3). They concluded that these choices were probably dependent upon a change in taste mechanisms (that is, they were tropism responses) rather than being a trial and error learning process, even though in most cases, it was 4-5 days before the rats were ingesting a high level of calcium lactate, and about 10 days before a plateau was reached.

However, a suggestion that feedback may be involved is

provided by an experiment carried out by Hellwald (1931) in which laying hens were placed on a calcium deficient diet. After 5 days they were offered either macaroni with fragments of eggshell concealed within, or of macaroni alone. The first group consumed on average 17 grams of calcium without tasting it, and when a few hours later both groups were offered crushed eggshell ad libitum, the first group ate 5 grams and the second group 19 grams. This finding may be contrasted with that of Falk and Lipton (1967), where it appears that in the case of sodium, even massive repletion does not impair sodium appetite.

Of course, Hellwald's experiment does not necessarily imply feedback, - calcium is rapidly absorbed, and the deficit might have been completely corrected by the time he tested their intake. It would be necessary to repeat his experiment, using a number of different delays, before offering the ad lib. eggshell. Wood-Gush and Kare (1966) suggested that feedback played a role in the selection of a calcium enriched diet, over one deficient in calcium.

The finding that parathyroidectomised rats show a preference for magnesium chloride is especially interesting in view of the report that parathyroid tetany can be prevented in dogs by the oral administration of magnesium chloride (Luckhardt, Waud and Brannon, 1926). These workers postulated that the magnesium chloride depresses the irritability of the central nervous system, thereby preventing the convulsive seizures which other-

wise occur. This finding in dogs implies that the hyperexcitability of the tetanic state is aversive, and that the ingestion of an agent which reverses this state is rewarding.

The calcium appetite of parathyroidectomised rats has been used to bioassay substances which affect blood calcium (Richter and Birmingham, 1941). It was demonstrated that appetite for a 2% solution of calcium lactate could be reduced to normal either by increasing the calcium content of the food, or by the administration of Vitamin D preparations which produce hypercalcaemia by the mobilisation of calcium from bone. The appetite could be reduced by administering parathyroid extract, but 100 units/day were required to bring it right down to normal, and this dosage was definitely toxic. These findings suggest that the factor responsible for the calcium appetite is the level of calcium in body fluids rather than a deficit in total body calcium.

Widmark (1944) found that calcium-replete rats exhibited a relative aversion for food enriched with calcium lactate in a 2 choice situation, eating about 35% of their intake in the form of the calcium-rich food. After 12 days of calcium deprivation, however, they now showed a slight preference (c. 60%) for the enriched food. He reports similar findings when calcium citrate was used in place of lactate, except that the preference was considerably more marked.

Scott, Verney and Morissey (1950) confirmed that rats

deprived of calcium by the feeding of a low calcium diet showed an appetite for diets containing this element. Surprisingly, the control rats also exhibited a preference for diets containing up to 6% of calcium carbonate. However, only the calcium deprived rats showed a preference for diets containing calcium phosphate and calcium sulphate, so it would appear that this appetite is a specific one, and not merely a preference associated with some other factor such as palatability.

Lewis (1964) has described behaviour resulting from calcium deprivation in parathyroidectomised rats, and concluded that calcium drive has properties common to other appetitive drives. Control rats drank small quantities of calcium lactate, with a peak at 0·3%, while the deprived rats drank much larger amounts at concentrations from 0·15% to 1·0%, continuing to consume some up to 3%. Five out of eight parathyroidectomised rats met the criteria for learning in an operant conditioning situation (with calcium lactate as the reinforcement) while none of the seven controls did so, and the calcium-depleted rats showed increases in lever pressing rates as the extent of calcium deprivation increased. In a later paper (Lewis, 1968) she compares this calcium drive with the similar drive already demonstrated for sodium (Lewis, 1960), and finds that during extinction trials in a Skinner box with 2 levers, rats did not demonstrate the ability to distinguish a calcium drive as they did a drive for sodium. These rats had been both parathyroidectomised and adrenalectomised,

and were able to maintain themselves in good health in their home cages, consuming sufficient quantities of the sodium chloride and calcium lactate solutions which were available. They had been trained to press the sodium chloride lever when they were sodium deficient, and the calcium lactate lever when they were calcium deficient, in each case the second lever being inoperative. They were then run with both levers operative, and alternately sodium and calcium deprived. Whether they were deprived of calcium or of sodium they pressed the sodium chloride lever more than the calcium lactate lever. She concluded that a calcium drive does not appear to have distinctive cues, and that calcium lactate does not have the rewarding properties of sodium chloride.

The results of this experiment are a little equivocal, and it may be premature to conclude that rats are utterly unable to discriminate a drive for calcium from one for sodium. It could be that under these conditions the sodium chloride is still more rewarding than the calcium lactate, even though the animals are ostensibly sodium replete and calcium deprived. The absence of distinctive sensory cues in the case of the calcium lactate cannot be a significant factor since the results were identical when the solution was marked with aniseed flavour. It undoubtedly appears paradoxical that a drive for calcium can lead to learning in a simple situation yet will not support adaptive behaviour in a complex one. Lewis concludes that perhaps over evolutionary time in rats no premium has been placed on a highly developed adaptive

mechanism for obtaining calcium since it is ubiquitous in the animal's natural habitat and would normally be consumed in adequate quantities without any active selection. She cites no evidence for this last conclusion, but the difference is unlikely to be physiological since both sodium and calcium are absorbed and retained by the rat with great efficiency, though it is true that body stores of calcium are very much greater, since large amounts of bone calcium can be mobilised if required.

Rodgers (1967) confirmed that calcium deprived rats show a preference for diets supplemented with 1·0% or 0·1% calcium chloride (though not for 0·01% calcium chloride). Rats which had been depleted and were then repleted with 1% calcium chloride in the drinking water also exhibited the preference. This result is reminiscent of that of Falk and Lipton (1967) but unfortunately there appeared to be no controls which had not been rendered calcium deprived. He then extended the experiment, depriving rats of calcium by feeding them Diet A, deficient in calcium, and then offering them a choice between Diet A, now calcium enriched, and Diet B, which was calcium deficient. Most subjects showed a strong and immediate preference for Diet B. Rodgers concludes that calcium deprivation results in preferences for novel diets, and this preference is greater than any specific unlearned preference for calcium that may also have been present in calcium deprived animals. This finding is very similar to that described by Rodgers and Rozin (1966) for thiamine deprivation in rats.

Some work has been carried out on the ability of chickens,

and in particular of laying hens, to balance their diet with regard to calcium when an insufficient amount is supplied in the food. Johnson (1954) offered foodstuffs, including oyster shell, to three different strains of birds, on a free selection basis, and found that the calcium intake of the light breed averaged 2·9%, and that of the heavy breed was 2·5%, while the hybrid was intermediate. These intakes are probably marginally sufficient.

Petersen et al. (1963), comparing White Leghorn layers, which received 2·25% calcium in their diet and ad libitum access to oyster shell, with controls which received 3·6% calcium in the food and no supplement, reported that the birds receiving the oyster shell supplement consumed on average a total of 4·375% calcium. Shell consumption was highest during the early laying period and there was no difference in eggshell quality between the two different treatments.

Mehring (1964) provided New Hampshire laying hens with food containing either 0·2% or 1·24% calcium and a calcite supplement with 33% calcium to which the birds had continuous access. The controls received 2·43% calcium in their food and no supplement. The birds receiving the supplement ingested a total of 3·45% and 3·70% of calcium respectively, substantially more than the National Research Council recommendation (2·75%). There was no difference between any of the three groups in egg production, efficiency of feed utilisation or hatchability, but the eggs from the birds with free access to calcite had greater eggshell strength.

A study comparing the relative intakes of 2 kinds of calcium supplement - oyster shell and calcite - in floor pens and

in individual cages, and at 3 levels of dietary content (1%, 2%, and 3%) has been carried out (Griminger and Lutz, 1964). A preference for the shell rather than the calcite was observed, and in the floor pens the intake of the supplement was closely linked to the dietary calcium levels. In individual cages the appetite for calcium appeared to be much higher, and did not fall as the level of calcium in the diet was increased. This may be because the eating of supplement is one of the few activities available to caged birds, while in pens intake is much more closely related to need.

Helder (1960) has also examined the relative acceptability of different types of calcareous grit. His subjects were North Holland cocks and hens and exhibited a definite preference for North Sea shell over oyster shell, while calcite grit was hardly eaten at all. When calcite only was available they did not ingest sufficient to meet their requirements. The birds however were not naive and before the experiment began they had had extensive experience of the North Sea shell only.

There seems to be little work on the intake of birds other than the domestic fowl, although in the case of hen pheasants Sadler (1961) reports that when, during egg production, the calcium level of the ration was reduced from 2·65% to 0·53%, the intake of grit rose by a factor of 4, and the percentage of limestone as opposed to non-calcareous grit rose abruptly, from about 60%-70% to approximately 90%.

There is an observation suggesting that not all birds are

able to adjust their calcium intake appropriately. Taylor (1969) observed that when Brown Leghorn hens were provided with a calcium deficient diet (0·15%) and a calcium supplement of cockleshell ad libitum, 2 birds out of 16 failed to ingest sufficient shell to maintain egg laying, and the same was true of 1 out of 16 in the case of a heavy hybrid.

In general, though, the available evidence suggests that most hens are well able to supplement their ration from a source of calcium when this is provided separately. The resultant figure of total calcium intake is rather higher than that normally recommended for an inclusive diet. This is by no means proof that there is a specific hunger for calcium in fowls, but it cannot entirely be a question of palatability because, on the whole, there is an association between intake and requirement, although as mentioned above, this association does break down at times.

Findings in field studies suggest that birds on occasion actively select sources of calcium. Collias and Saichuae (1967) examined the crops and gizzards of Red Jungle Fowl in Thailand and found that about one third contained fragments of snail shell - a source of calcium in a habitat otherwise rather poor in this element. Harper (1963) reported that the grit obtained from the crop and gizzard of juvenile pheasants altered in composition as the age of the birds increased - the calcium content from birds of 6-8 weeks being 4·9%, and that from birds of 16 weeks and older being 0·3%. He suggested that the birds select

calcareous grit according to their physiological needs. In support of this he also (Harper, 1964) demonstrated an annual cycle in the intake of calcareous grit. The level of calcium in grit taken from the gizzards of wild hen pheasants was very low in winter, but when the nesting season began it rose steadily to a level of 3·0% and then fell again to low levels (0·05%) by the middle of the incubation period. A further rise occurred during the autumn to a peak of 2·6% in October. He accounts for this in terms of a recrudescence of reproductive activity, since there was no corresponding rise in juveniles.

Wood-Gush and Kare (1966) have examined this calcium appetite of chickens in greater detail in order to establish whether or not it is a true specific hunger. Growing broilers were fed on calcium deficient diet from the age of 3 weeks until blood calcium levels were significantly below those of controls, and then both groups were given a choice between the calcium deficient diet and the same diet with the addition of calcium carbonate. Both groups showed an aversion to the calcium enriched diet, but the deprived group took 33% of their intake in the form of the enriched diet, significantly more than the controls, which took 22%. When the two groups were given a choice between water and 2% calcium lactate, they showed a strong aversion to the calcium lactate, only 15-20% of their liquid intake being in this form, and there was no difference between the two groups. The authors conclude that this appetite for calcium may not be a true specific hunger (in the sense that no learning is involved),

but that the chickens may learn to recognise the corrective nutrient after a period of experience which is related to the palatability of the nutrient and the degree of deprivation.

6. APPETITE FOR VITAMIN B.

Richter et al. (1938) reported that Vitamin B deprived rats showed an aversion to protein and carbohydrate and a craving for fat, which they explained in terms of the altered metabolism of these substances during Vitamin B deprivation. When given access to yeast, which is an excellent source of Vitamin B, the animals consumed it eagerly, and corrected the deficiency. In a later paper (Richter and Barelare, 1939) they demonstrated that when thiamine only was given there was a dramatic switch, sucrose being consumed almost exclusively. If thiamine, riboflavin, nicotinic acid and pyridoxine were offered separately to Vitamin B deprived rats they showed an active appetite for each of the vitamins, the fat appetite decreased to normal, and the sucrose appetite rose to normal, but the appetite for casein remained depressed (Richter and Hawkes, 1941). A possible explanation for this is that growth remained poor and so the requirement for protein continued to be low.

Harris, Clay, Hargreaves and Ward (1933) made the first comprehensive study of an appetite for a single dietary factor. Rats which were depleted of thiamine and were then offered 2 foods, one of which was thiamine-free while the other was supplemented with thiamine, almost invariably selected the thiamine-rich

food while controls ate the two foods indiscriminately. The animals could not make the appropriate selection if:

1. The thiamine content of the enriched food was less than adequate.
2. The thiamine content of the enriched food was much more than adequate - presumably because they could ingest sufficient thiamine under these conditions when making a random choice.
3. Too many foods (6-10) were offered as choices. However, even if many foods were offered for choice, but the rat was allowed to eat only the thiamine-containing food for a short period so that it experienced its beneficial effects, the animal would eat no other food choices thereafter, and its appetite was considered 'educated'. They found that if a synthetic (tasteless and odourless) vitamin was used rather than a natural source, the rat was initially unable to differentiate, but could readily learn to associate the presence of the vitamin with an added flavour. They concluded that the ability of the Vitamin B depleted rat to discriminate between diets containing the vitamin, and those deficient in it, depends not on vague instinct but on an association between the distinctive character of the diet (smell, taste or appearance) and on experience of the prompt beneficial effect e.g. appetite, alimentary tone, and others, which follow on its consumption. With certain other dietary essentials, where the benefit is less immediately apparent, the rat is unable to distinguish between the deficient and the adequate diets.

This finding has been confirmed and extended by Scott and his co-workers. They found that when rats were deprived of the

appropriate vitamin for 3 weeks, then offered a choice between a vitamin-free and a vitamin-enriched diet, 10 rats out of 10 chose that supplemented with thiamine, 8 out of 10 chose the pyridoxine-enriched diet, 6 out of 10 ate the riboflavin-rich diet and no preference at all was shown for calcium pantothenate. This may be ascribed to the fact that the first three vitamins have a characteristic taste, colour or odour, whereas calcium pantothenate has not, and when it was artificially flavoured with aniseed the total intake was much higher, although some animals still failed to choose it (Scott and Quint, 1946a). In a later experiment the three vitamins thiamine, riboflavin and pyridoxine were presented, linked with a physical property of the diet (aniseed flavour) and when a preference was firmly established the flavour was switched from the enriched to the deficient diet. In each case the subsequent preference was clearly linked to the flavour rather than to the vitamin (Scott and Verney, 1947).

Some additional work was carried out on this subject by Tribe and Gordon, who found that non-deprived rats showed a preference for diets enriched with Vitamin B, although this preference was much more marked in the case of deprived rats (1953a). Rats were unable to choose a beneficial diet where the feedback was considerably delayed. They were given a choice between two diets. One contained starches which permitted intestinal synthesis of Vitamin B, but was unpalatable, while the other was palatable but could not be utilised for synthesis. All the rats chose the palatable diet and died (Tribe and Gordon, 1953b).

7. THIAMINE APPETITE.

Adaptive behavioural changes, especially those involving selection of foods, have been more thoroughly investigated in thiamine deficiency of the rat than in any other vitamin deficiency, largely due to the work of Rozin and his co-workers. The analysis and understanding of the mechanisms of thiamine specific hunger is more advanced than for any other dietary essential, with the exception of sodium. Rozin, Wells and Mayer (1964) confirmed the result obtained by Scott and Quint (1946a) in demonstrating that thiamine deprived rats exhibited a marked preference for diets containing thiamine in all concentrations over diets deficient in thiamine. However, they failed to show any preference for thiamine solutions as opposed to water, and indeed the strongest solution of thiamine appeared to be aversive. Even when the solution was labelled with a distinctive neutral flavour the performance of the animals did not improve, indicating that they were not failing to select it because they were unable to identify it. Since ingestion of all but the weakest of the solutions offered was followed by rapid recovery from the deficiency, the authors concluded that an explanation of thiamine specific appetite in terms of simple need reduction (Harris et al., 1933; Scott and Verney, 1949) was inadequate.

This finding may be compared with that reported by Luria (1953) who observed the intake of thiamine solutions by thiamine-depleted and by control rats in both free intake and operant conditioning situations. Under free intake conditions both groups

showed an aversion to thiamine as opposed to water, but at the two highest concentrations of thiamine (0·25 and 0·025%) the depleted group drank consistently more than the controls, who tended to decrease their intake following prolonged contact with thiamine. One might say, therefore, that in this case the deprived animals exhibited a relative preference. In the Skinner box, lever pressing for thiamine solution, the rate of pressing was significantly higher for the depleted group for the first hour, but the two rates tended to merge thereafter, and there was no difference after 18 hours. The author described these results as "disquieting" in that the greatest difference between depleted and control animals preceded the earliest measured physiological effects of thiamine. The increased rate of bar pressing in the early periods could be ascribed to enhanced non-specific activity in the deprived animals. One must conclude that these results, though somewhat anomalous, offer no support to a learning theory of specific appetite, - at least in the case of thiamine. Richter (1956) reported selection of thiamine-rich solutions by deprived rats, but was later unable to repeat these results (Rozin, Wells and Mayer, 1964). These latter authors discarded the need reduction hypothesis on the grounds that the rats were ingesting substantial quantities of thiamine solution followed by recovery, and that the hypothesis would demand that the response leading to these beneficial effects, namely the ingestion of the thiamine solution, should be reinforced.

Rozin (1967) noted that the anorexia associated with thiamine deficiency in rats disappeared when a new food was offered, the animals consuming it eagerly, whether it contained thiamine or not. This observation was confirmed by Rodgers and Rozin (1966), who demonstrated that thiamine deprived rats offered a choice between a novel diet and a familiar diet invariably showed a marked preference for the novel diet. If the novel diet contained thiamine this preference was maintained over a 10 day period. If the novel diet was thiamine deficient and thiamine was added to the familiar diet at the beginning of the choice test, the rats switched from an initial novel diet preference, which lasted 3 to 4 days, to a familiar diet preference which was maintained to the end of the test. Control rats showed no marked preference for either diet. The authors speculated that the exclusive initial ingestion of a novel diet may facilitate the development of a sustained, learned preference. This gives the greatest possible opportunity for reinforcing the ingestion of the most beneficial diet. This behavioural characteristic is described as "neophilia".

Rozin and Rodgers (1967) confirmed that rats deficient in thiamine, riboflavin and pyridoxine, unlike controls, all showed strong preferences for novel diets, and that this preference was also exhibited when the animals had first received injections of the appropriate vitamins so that they were no longer deprived. This finding is in agreement with a report by Rozin (1965) that a

past history of thiamine deprivation appears to be a sufficient condition for the development of thiamine preference, even though the thiamine status of the rats is normal at the time of testing. These results led the authors to postulate the existence of "palaeophobia" - a learned specific aversion to vitamin deficient diets.

In this context a finding by Rzoska (1953) takes on an added significance. When testing the reaction of domestic rats to foods which on an earlier occasion had contained poison, he reported a range of approaches. Some rats refused to consume any food (after 24 hours of food deprivation), some ate a little after hesitating for a while and others accepted it quite freely. In addition he observed that besides this attitude, clearly directed against a special bait, there was in some cases a change of behaviour even towards plain food, illustrated by the cautious approach of some rats to bread paste, after an experience with an entirely different poison bait a week before. These findings suggest palaeophobia (described by Rzoska as "bait shyness") and in addition foreshadow Rozin's concept of generalised neophobia.

These concepts of palaeophobia and of neophobia were extended by Rozin in later papers (1967, 1968b). Palaeophobia is demonstrated by:

1. Spillage of the familiar deficient diet (normal rats may spill highly unpalatable foods). However a note should be added here that others (Tribe and Gordon, 1953a) failed to observe any such tendency in Vitamin B deficient rats.

2. Redirected feeding responses (chewing inedible objects, licking the cage walls and floor) in the presence of deficient diets.

3. After recovery on a new diet, an avoidance of the familiar deficient diet, even when the rats are food deprived with no other food available.

In this complex and interesting study, Rozin compared the effects of slow thiamine deficiency produced by dietary insufficiency, rapid thiamine deficiency produced by an antivitamin (oxythiamine hydrochloride) and rapid poisoning produced by the ingestion of lithium chloride, examining the effect of these three procedures upon dietary preferences, in both domestic and semi-wild rats. In all cases the experimental design was similar. A rat was fed Diet A for a period and was then given a choice between Diet A, Diet B and Diet C. Diet A might have one of the deleterious effects listed above in the case of the experimental groups, but in the case of controls would be entirely innocuous. Diet B was a familiar diet which the rat had previously ingested and knew to be "safe", and Diet C was an entirely novel diet. The results make it clear that both the vitamin deficiency and the poisoning produce learned aversions (palaeophobia) for the diets associated with these conditions. The three experimental groups differed very little in terms of the learned aversion which developed, all groups ingesting almost the minimal possible amount of Diet A, the harmful diet. In addition, compared with controls, all the experimental groups showed an enhanced neophobia, or rejection of

the novel diet, Diet C. When the results for the two strains of controls are compared it is plain that the semi-wild group shows a neophobia, or suspicion of new food, even in the absence of an aversive experience, while the domestic group actually shows a slight neophilia. This is hardly surprising - adjustment to domestication must involve a minimisation of neophobia. However the increase in neophobia consequent to poisoning or to deficiency is about the same (about 40%) in both domestic and semi-wild rats.

The implications of these findings are difficult to assess. It may be that poisoning triggers a pre-existing but latent neophobia, which is already partly expressed in wild rats, or it may be that the acquisition of a generalised aversion to new foods follows a single aversive experience with one new food. Rozin suggests that there is now no necessity for invoking a switch to neophilia in order to account for the expression of specific appetites. He concludes that specific appetites (other than that for sodium) and poisoning responses are fundamentally similar, and consist of a learned (conditioned) aversion for the harmful diet (palaeophobia) and an increased suspicion of novel diets (neophobia). These two factors co-operate in directing the animal towards eating a familiar, safe food. The apparent neophilia reported in earlier work (e.g. Rodgers and Rozin, 1966) is in fact an expression of the relative strengths of palaeophobia and neophobia. When only 2 diets are available, the palaeophobia

for the harmful diet may be so intense that the neophobia for the novel diet may be entirely overcome.

However, it does not seem possible to discard altogether the concept of neophilia in view of Rozin's own account (1967) of thiamine deficient rats "showing immediate, sustained and vigorous feeding" on a novel diet as soon as it becomes available. This kind of behaviour hardly suggests the overcoming of an intensified neophobia. Nor does it seem possible to discard entirely the hypothesis of need reduction and learning as it relates to specific hungers. It is true that the failure of deprived rats to select thiamine-rich solutions is difficult to account for in terms of the need reduction mechanism, but it is also true that Rozin's palaeophobia-neophobia theory in its simplest form cannot explain the tendency of rats to switch back to a diet for which they have a learnt aversion when the novel diet which was apparently so attractive initially, has no positively reinforcing value. It may be that an integration of these two hypotheses will be best able to account for all the extant facts.

8. PROTEIN AND AMINO ACIDS.

Some findings relating to the self selection of protein have been cited above. Early workers reported that animals were able to select diets adequate in protein. Osborne and Mendel (1918) demonstrated a preference in rats for a diet containing 18% casein rather than for a protein-free diet, while a preference for a diet with the complete protein lactalbumin, rather than the

incomplete protein zein, was described by Mitchell and Mendel (1921). Richter, Holt and Barelare (1938) reported adequate intake of casein by rats under a self selection regime.

On the other hand, most of the later investigators found that their experimental animals were unable, in greater or lesser degree, to balance their ration with adequate amounts of protein. Kon (1931) found that rats under a self selection regime consumed a diet with only 6-9% of protein, and grew very poorly, while Scott (1946a) concluded that about two thirds of his experimental rats found casein palatable and ingested adequate quantities, and that one third found it unpalatable, rejected it and died. Pilgrim and Patton (1947) independently reported precisely similar results. Animals offered a choice among four different proteins of different nutritional value did not respond in a consistent manner. Scott concluded that "nutritional need for protein bears a poor relationship to the selection of protein", and that the selections observed were most likely due to simple preferences, (Scott, 1946b). McDonald, Stern and Hahn (1963) reported that only 50% of individually housed animals ingested adequate amounts of casein from the very beginning of the self selection procedure, although the performance of the remainder improved with time. Rogers and Harper (see Harper, 1967) carried out an incomplete study which suggested that if rats are offered a choice between two diets that differ substantially in protein content, they show preferences for high protein (50% casein) over low protein (9%

casein), and for moderate protein (25%) over high protein (50% or 80% casein). The authors emphasised that this work needs repeating.

With regard to the dietary adjustment of protein intake in response to an increased requirement, Richter and Barelare (1938) found that the intake of protein increased under self selection conditions in pregnant and lactating female rats, suggesting that an effective homeostatic mechanism was operating. However, later work has failed to reproduce these findings. Scott, Smith and Verney (1948) found that pregnant female rats increased their total food intake but the proportion of casein ingested did not rise, and following parturition many pups were lost to cannibalism. Tribe (1955) reported that female rats selected diets deficient in both calories and protein while pregnant and lactating, and in this case too, many of the young rats were eaten, again suggesting that the diet consumed was in some respects inadequate. Gordon and Tribe (1951) found that the protein intake of pregnant sheep was low, resulting in heavy ewe and lamb mortality. Tribe (1950) reported that the protein intake of sheep on a self choice system is dependent mainly upon the nature and palatability of the protein - being approximately double the amount necessary when the protein source is linseed cake meal, and yet inadequate when they are offered white fish meal. As regards birds, Kare and Maller (1967) describe preliminary experiments in which protein-deprived chickens failed to select a 1% solution of casein in a two choice situation.

Some of the more recent work has concentrated on the effect of a deficiency of a single amino acid rather than of protein as a whole. Beadles, Braman and Mitchell (1930) fed to rats two diets identical except that one was balanced while the other was deficient in cystine. The animals did not distinguish between the two diets, as judged by the quantities consumed in paired feeding, where one rat received the deficient diet and its control the complete diet.

Later investigators however have demonstrated that the omission of an amino acid from a diet can have a profound effect on appetite. Frazier et al. (1947) described studies in which the omission of any one of nine essential amino acids from an otherwise complete diet resulted in a marked decrease in the food intake of rats within 24 hours, while the replacement of the amino acid resulted in a stimulation of food intake within 24 hours, whether the missing amino acid was included in the diet or administered parenterally.

Harper (1967) reviews the literature, and concludes that variations in the concentrations of dietary or plasma amino acids are unimportant in the regulation of food intake in the normal organism consuming a normal diet: but that appreciable deviations from normal in amino acid intake, dietary amino acid pattern or plasma amino acid pattern may set into action subsidiary regulating mechanisms that can affect both food intake and food preference. Whether the effects observed can legitimately be

classed as specific appetites, he writes, is problematical.

Sanahuja and Harper (1962) discuss the behaviour of protein depleted rats given a choice between two diets, one being an imbalanced diet lacking histidine and supporting growth, while the other was a protein free diet which would not support growth at all. For 3 days the animals selected the imbalanced diet, but thereafter preference shifted to the protein-free diet. After they had lost weight they again consumed some of the imbalanced diet, following which there was once more a switch to the protein free diet. These periods of alternative preference are repeated, in somewhat rhythmic form, but towards the end of the experiment the shifts in preference become progressively less extreme. If the imbalanced diet is corrected by adding 0·1% of histidine hydrochloride this behaviour is not observed, and a preference for the corrected diet is seen throughout the entire experiment. The authors conclude that their results cannot be adequately explained in terms of the differential palatability of the two diets, but that the experimental rats are maintaining a balance between two aversive states - the overall protein depletion produced by the protein-free diet, and the abnormal plasma amino acid pattern produced by the imbalanced diet. Finally, an equilibrium in the selection of the two diets is reached at some stage of partial depletion.

When protein-depleted rats were offered a choice between the imbalanced diet and the corrected diet (which contained an

additional 0·1% histidine hydrochloride), on the first day they ate 53% of their food intake in the form of the corrected diet, on the second day 78%, and by eight days it was averaging over 90% (Sanahuja and Harper, 1963). The food containers were placed at random in the cages. When the same experiment was carried out with non-depleted rats, the corrected diet this time containing only 0·05% histidine hydrochloride, the results were very similar, comparable figures for the first two days being 24% and 43%, but again by 8 days around 90% of their intake was made up of the corrected diet. This suggests that even in rats of normal protein status the imbalanced diet very rapidly has an aversive effect. The authors conclude that under these conditions, the rat has a remarkable ability to select the more beneficial of two diets. The development of the preference over time suggests that there is a learning process involved in this selection. It is interesting that humans appear to be unable to select a balanced mixture of amino acids in preference to one lacking methionine and phenylalanine (Bowering, Margen and Calloway, 1969).

Rats sometimes fail to achieve a satisfactory balance. In another experiment (Harper and Rogers, 1965) rats were given a choice between protein-free diets and diets with an imbalanced amino acid mixture, which lacked either threonine or tryptophan. Some rats died from protein malnutrition after 30 days of eating the protein-free diet when they had at all times access to the imbalanced diet which, if eaten in moderate quantity, would support

a moderate rate of growth. As threonine was added back to the imbalanced diet, increased quantities of it were eaten with each increasing increment. This behaviour, although at first sight paradoxical, may have adaptive features, since it is known (Sidransky and Farber, 1958) that if rats are force fed on a diet that is devoid of one amino acid (threonine, methionine or histidine) they will develop pathological lesions and survive for only a short time; whereas if they are allowed to eat the same diet ad lib. their food intake will fall, no pathological lesions develop, and they will survive longer. The mass action effect of the liver, synthesising protein, depletes the level of the limiting amino acids in the blood, and it is postulated that this acts as a signal to some centre which controls appetite.

Direct evidence has now been obtained relating to this control (Leung and Rogers, 1969). Rats were fed a diet high in all the essential amino acids, with the exception of one - either threonine or isoleucine. As predicted, their food intake was 40%-50% below that of controls. When they were constantly infused with minute amounts (4 mg. per 24 hrs.) of the growth limiting amino acid into the carotid artery, the food intake was restored to normal, while infusion of the same amount into the jugular vein did not prevent the marked reduction of food intake. These results provide evidence for a food intake regulatory function of some portion of the brain which is sensitive to the concentration of the growth-limiting amino acid in blood.

The effect of an amino acid excess upon dietary selection has been examined in the case of leucine (Harper, 1967). Rats offered a choice between a high leucine diet and a protein free diet will select almost exclusively the protein free diet for as long as three weeks. If they are offered a choice between a high leucine diet and the high leucine diet to which has been added additional isoleucine and valine to correct the leucine-isoleucine and valine antagonism, after two or three days they select the diet containing additional isoleucine and valine almost exclusively, and gain weight. However, given a choice between the leucine, isoleucine and valine (LIV) diet and the protein-free diet, they eat mainly the latter, and slowly lose weight. Adding other amino acids to the LIV diet does not result in a clearcut choice of the improved diet over the protein-free diet, and it thus appears that the rat has a specific aversion to a high leucine diet even when it is improved nutritionally so that it will support a fairly rapid rate of growth.

9. OTHER SPECIFIC HUNGERS.

A certain amount of work has been carried out to establish the existence or otherwise of a specific hunger for a number of other vitamins and minerals.

Potassium

Richter, Holt and Barelare (1938) on the basis of the results of an extensive self selection procedure, concluded that rats exhibited a specific hunger for potassium (as the chloride).

However, Scott, Verney and Morissey (1950) found that rats made potassium deficient by feeding a low potassium diet neither preferred nor avoided diets enriched with potassium dihydrogen phosphate when compared with controls, in a two choice situation.

Magnesium

Scott, Verney and Morissey (1950) showed that magnesium deprived rats, when compared with controls in a two choice situation, actually avoided diets enriched with magnesium oxide, carbonate or sulphate to such an extent that they succumbed to hypomagnesaemic tetany in the presence of ample quantities of magnesium. It is difficult to explain such apparently maladaptive behaviour, but it is suggested that since magnesium deficiency appears to produce a state akin to euphoria, and that this is reversed by dietary magnesium, which is a central nervous depressant, the rats are in fact exhibiting a learned avoidance, because the deficient state is more rewarding than the normal.

Zinc

Humphries and Quarterman (1968) deprived rats of zinc by feeding a zinc free diet, and then offered them a choice between the zinc-free diet and the same diet with the addition of 6 p.p.m. of zinc. (The particular zinc salt used was unspecified). The rats showed a preference for the high zinc diet, and this was greatest on the first day of choice, and then progressively declined, until by the sixth day they were eating equal amounts of both diets. In addition, these zinc deficient rats exhibit a

preference for a zinc deficient diet which is protein free, rather than a zinc deficient diet with normal levels of protein. 100 micrograms of zinc per rat, given orally or intraperitoneally, did not affect diet selection for at least 5 days. When zinc deprived rats were given a choice between two protein free diets, one with added zinc and the other zinc deficient, there was no discrimination. Thus no preference for zinc is observed in the absence of protein, and clearly the metabolism of these two dietary components is closely linked. The authors compare these findings with those of Harper and Rogers (1965), who found that rats prefer a protein-free diet to one which leads to an imbalance of plasma amino acids, and they emphasise that the zinc deprived rat also has suffered an imbalance in plasma amino acids. However, although an inadequate amount of experimental detail is given in this paper, it does appear that part, at least, of their results can be explained by invoking Rozin's hypothesis of palaeophobia. This is supported by their finding that, in a single choice situation, zinc deficient rats eat more of a high zinc diet than rats which are offered a zinc-free diet, and that this difference appears after only 4 hours, and gets progressively greater. It could also perhaps be explained by postulating a return of plasma amino acids to a normal balance when zinc is ingested, thus leading to an increase in food intake (Leung and Rogers, 1969), but the time course seems almost too rapid for this to have occurred.

Turkeys too are able to select diets supplemented with zinc (Vohra and Heil, 1969). Groups of day-old poults were given

a choice between a zinc-deficient diet and the same diet supplemented with 100 ppm of zinc. 80% of the birds showed a preference for the enriched diet by the time they were 14 days old. They were unable to select the zinc if the feeders were randomised daily.

Vitamin A

Over a period of 60 days Price (1929) offered a group of 40 chicks a choice of three different butters, their positions in the cage being randomised daily. They consumed 415 grams of butter high in Vitamin A and D, 289 grams of that high in Vitamin A and low in D, and 209 grams of that low in both A and D. An examination of the three butters gave no clue as to the means by which they made their selection and he concluded that the chickens appeared to be able to select a food higher in vitamins, according to their needs.

Harris et al. (1933) report very briefly that rats depleted of Vitamin A were offered a choice between a diet deficient in Vitamin A and one containing an adequate amount of the vitamin, but did not exhibit any ability to select the adequate diet. Unfortunately no details concerning length of deprivation or length of choice period are given.

Jukes (1938) fed chicks on a diet deficient in Vitamin A, and allowed them free access to alfalfa meal, which has a bitter flavour to humans, but is rich in Vitamin A. The chicks did not consume sufficient to avoid a sub-acute Vitamin A deficiency, and he concluded that birds do not select a balanced diet 'instinctively'.

This experiment was repeated over a longer time scale by Harriman (1955) who offered Vitamin A depleted rats a choice between the deficient test diet and alfalfa meal. For 14 days their intakes were comparable with those of controls, but thereafter the depleted animals consumed progressively larger quantities of the Provitamin A rich supplement, and corrected the deficiency. Harriman interprets this as evidence of learning - well within the context of Hull's theory of secondary reinforcement. In view of the fact that alfalfa meal is bitter, and normally highly unpalatable, a finding of Bernard, Halpern and Kare (1961) takes on an added significance. They reported that rats in an advanced stage of Vitamin A depletion showed abnormal taste responses. After several weeks deprivation, a marked decrease in rejection of quinine and in selection of sodium chloride solutions occurred. Administration of Vitamin A resulted in rapid recovery of sodium chloride selection but not of quinine rejection. These data suggest that Vitamin A may have a direct effect in the two sensory modalities - salt and bitter - tested. In the case of rats, at least, it could be that loss of ability to taste bitter substances permitted increased intake of the Vitamin A rich supplement, and that this tendency was then positively reinforced, thus accounting for the lengthy delay in Harriman's experiment between the supplement becoming available, and its being consumed in any quantity.

Vitamin D

Very little work has been carried out investigating a

preference for this vitamin, but what little evidence there is suggests that there is no specific appetite for Vitamin D.

Harris et al. (1933) reported that Vitamin D depleted rats showed no preference in a two choice situation for a diet containing an adequate quantity of Vitamin D over a diet deficient in it. No experimental details were given. Young and Wittenborn (1940) offered normal and rachitic rats a choice between cotton-seed oil and cotton-seed oil plus Vitamin D. Neither group showed any preference for the Vitamin D supplement, either on brief or on prolonged exposure.

One experiment has been performed to examine the behavioural effects of an excess of Vitamin D. Harris and Moore (1928) found that rats given a single, massive, toxic dose of Vitamin D showed an aversion to diets containing irradiated ergosterol (Vitamin D), whereas they ate a diet containing non-irradiated ergosterol very freely. They point out that adequate control experiments were not performed, and that the results could be ascribed to distasteful impurities in the irradiated ergosterol. Nevertheless, this result is interesting in the light of Rozin's (1967) results.

Phosphorus

Wilder (1937) maintained rats for 30 days on a diet high in calcium and deficient in both Vitamin D and phosphorus ("r" diet) in order to produce rickets, and then offered them a choice between the "r" diet and the same diet with the addition of 1·7% sodium hydrogen phosphate ("a-r" diet). None of 27 rats exhibited a preference for the "a-r" diet (which should have been anti-rachitic).

Controls maintained on a balanced ration, with adequate Vitamin D and phosphorus, showed no discrimination between the "r" and the "a-r" diets. When rats were maintained on the "r" diet for only 4 days, and then offered a choice, 68% showed a preference for the "a-r" diet, while of controls fed the "a-r" diet for 4 days 71% selected the "r" diet. These latter results may be highlighting an effect of neophilia, but the earlier results at first sight appear to demonstrate an inability to select phosphorus while in a state of need. It is possible, however, that the concomitant deprivation of Vitamin D may very well reduce the absorption of the phosphate in the "a-r" diet and prevent its deposition in the bones, particularly in view of the high concentration of calcium in the diet. In fact, therefore, the "a-r" diet may not be a source of available phosphate at all.

Green's report (1925) of osteophagia in phosphorus deprived cattle is a field observation, while as described above Gordon, Tribe and Graham (1954) failed to demonstrate an appetite for phosphate supplements in phosphorus deprived cattle and sheep under extensive husbandry conditions, although Stoddard and Mickelson (1961) did find an appetite for phosphate supplements in cows under more intensive conditions.

Thus the literature fails to yield a single experiment where phosphorus deprived animals are offered a simple choice between two similar diets, one adequate and the other deficient in phosphorus, and until this is carried out the question of the existence of a specific appetite for phosphorus in any species

must to some extent remain an open question.

10. FOOD PREFERENCES IN THE FOWL.

A number of studies have been carried out in the domestic fowl, investigating its food preferences. In this context its sense of taste is important, since preferences for food will clearly be closely linked to this sensory modality, but other modalities such as vision and tactile sense will also be implicated.

Engelmann (1940) investigated form perception in hens, and concluded that the grain preference of the fowl was based upon shape and colour rather than taste. Adult hens preferred grains shaped like wheat rather than like rye - even when the "wheat" grains had actually been formed from rye meal. Naive chickens which had never seen grain before preferred elongated shapes such as grains to round objects like peas (Engelmann, 1942). Initially they selected rye, but soon, like the adult fowls, came to prefer grains of wheat.

Fantz (1957) examined form preferences in newly hatched chicks, and he reported that they consistently pecked more at round than at angular objects. This preference was maintained after intensive unrewarded pecking, though it could be increased or decreased by appropriate reinforcement, namely the presentation of their food as either round or angular pellets. Thus Fantz concluded that there is a strong innate component present in the chicken's preference for certain forms, but that it can be modified to a certain extent by experience.

In man, tastes are of course classified into four main groups, - sweet, acid, salt and bitter, - and attempts have been made to establish whether the chicken too can distinguish these four classes. Engelmann (1934) observed that the fowl would discriminate in choices between acids, salts, sweetness and bitter, and he was of the opinion that fowls showed a strong preference for sugar solutions, as do many mammals. Jacobs and Scott (1957) confirmed that 6 week old chickens exhibited a strong preference for 12% sugar solution rather than water, and that this preference was still present following the forced feeding of large quantities of glucose solution. Kare, Black and Allison (1957) also described a moderate preference for sucrose.

On the other hand Kare and Medway (1959) expressed a contrary view - demonstrating that the fowl is indifferent to glucose and sucrose over a wide range of concentrations, but rejects xylose, which is toxic but nevertheless is selected by many mammals. They claim that the pattern of discrimination suggests that sweetness as recognised by man is of no consequence to the fowl.

This question cannot yet be regarded as finally settled, since work on broiler chickens in progress in this department (Gentle, 1969) has indicated an apparent preference in favour of mash containing 20% sucrose over a control mash without sugar. In addition, Kare and Halpern (1961) found that, although chicks on an ad lib. diet exhibited no preference for a 10% sucrose solution, when their food intake was restricted their total fluid

intake almost doubled and a marked preference for the sugar was observed. This finding can, of course, be explained as a reflection of energy homeostasis rather than as a palatability response, but it would appear that in any case the sugar can acquire secondary reinforcing properties, since when the birds were returned to an ad lib. diet there was still, at least for a time, a preference for sucrose. This question has been examined still further by Kare and Maller (1967). They found that Jungle Fowl, unlike the domestic variety, exhibited a preference for 10% sucrose rather than water in a two choice situation, although this preference was slight compared with that seen in rats. When fed a diet diluted with cellulose, both species of fowl increased the proportion of sucrose consumed, and also their intake of fluid. When an enriched diet was presented, the jungle fowl reduced their food intake, but neither group rejected the sugar solution under these conditions when they were receiving an intake of calories in excess of their needs. The authors suggest that domestication has produced an animal with diminished sensitivity to caloric regulation; however, when their results are carefully examined the superiority of the jungle fowl in this respect is by no means striking. Another worker (Morris, 1968) has reviewed the evidence for the ability of fowls to adjust their food intake appropriately when diets with different levels of energy are fed, and concludes that this adjustment is imperfect in the majority of cases. Birds fed high-energy diets usually over-consume calories and gain more in weight than birds fed lower energy diets.

There is a strain difference, and light hybrids compensate more efficiently than heavy hybrids which have a characteristically higher energy intake.

Rats show an inverted 'U' response with regard to the selection of sodium chloride, the concentration for maximal acceptance being about 0·9%, but it would seem that insufficient work has been carried out on this problem in the fowl to be able to state with confidence how it would respond to any particular concentration.

Kare and Biely (1948) recorded the intake of salt solution and water offered to young chicks on alternate days. There was no difference between the relative amounts of each solution consumed when 0·9% NaCl was offered, but 1·8% NaCl was strongly rejected. Kare, Black and Allison (1957) offered 2% NaCl and water in a two choice situation, and again the salt was rejected.

Duncan (1962) has carried out an extensive study of salt preference, mainly in the feral pigeon, but also in bantam cocks and six week old growing chicks. Using the method of single stimuli, where the solution is presented for 2 hours, following overnight fluid deprivation, various concentrations of salt were compared with tap-water. Pigeons yielded a preference curve very similar to that seen in rats, with the maximum preference point at 0·6%, all concentrations above 1% being aversive. The fowls were tested with 0·5% and 0·6% NaCl only, and in both cases a significant rise in consumption relative to water was observed, the greater preference being expressed for the 0·5% solution.

Using a simultaneous choice situation Engelmann (1934) reported that all solutions over 1·2% were aversive, and that there was no preference for weaker solutions. Kare and Ficken (1963) reported no apparent preference or rejection for NaCl solutions within the range 0·1% - 1·0% but it is not clear whether they were using a simultaneous choice technique or the method of single stimuli. One must conclude therefore that it is not yet established whether chickens exhibit a preference or indifference for weak solutions of sodium chloride, but it may be that the conflicts between the different reports can be explained in terms of the methodology of the various experiments. All the findings appear to indicate that the chicken rejects NaCl solutions when the concentration exceeds about 1·2%.

Kare and Ficken (1963) also tested the responses of chicks to a wide range of other salts, and found a variety of reactions. They were indifferent to some sodium salts at all concentrations from 0·1% - 1·0%, for example the acetate, sulphate and phosphate, others such as the bicarbonate and benzoate they accepted at low concentrations as readily as water, but rejected at the higher concentrations, while still others were rejected at all concentrations, for example the carbonate and cholate. A similar picture was seen when a series of chlorides was presented. Indifference was shown to magnesium and choline chloride, strontium and ammonium chloride were aversive at the higher concentrations only, while cupric and ferric chloride were rejected at all concentrations. Ficken and Kare (1961) had, however, found that there

was a considerable variation between different individuals. In the case of calcium chloride, for example, some individuals discriminated at concentrations only one tenth as great as others. The distribution of thresholds appeared to be of a continuous type, and no individuals appeared to be 'taste-blind', since all birds responded if the concentration reached a high enough level. Williamson (1964) has carried the study of individual variation to three generations. 200 fowls were screened for ferric chloride thresholds, and breeding was carried out with two groups, - those with the highest and those with the lowest thresholds being mated together with each other. This process was repeated for another generation, and then the ferric chloride thresholds of the two lines were tested and found to be significantly different, indicating that in the fowl they have a genetic basis.

The reaction of chicks to changes in the pH of their drinking water has been tested (Fuerst and Kare, 1962) in a 2 choice situation against water. In general they will accept solutions of strong mineral acids and bases ranging from pH 2 to pH 11, indicating a considerable degree of tolerance. In fact they exhibit a moderate preference for mildly acidic solutions over water. The authors speculate that this may be of adaptive significance in that it would have permitted the exploitation of a temporarily available food source of a strongly acidic nature.

Kare, Black and Allison (1957) have examined the response of chicks in a 2 choice situation to a range of bitter substances,

among others. They found that colocynth, a bitter taste to man, is acceptable to the chick, while cocillana, alfalfa and quinine, which are also bitter, were rejected.

It thus appears, that although the chick will respond to the flavours which humans describe as sweet, salt, acid and bitter it does so in ways which suggest that this classification is meaningless in the avian context. One further example which emphasises this point is the response to saccharin. Humans recognise this flavour as sweet and rats prefer it to water, but the fowl rejects it at a very low concentration, even though it accepts a similarly tasting (to man) sugar solution (Jacobs and Scott, 1957). It thus follows that one cannot predict how a fowl will respond to a particular food without carrying out an empirical test. The palatability of the food in other species may not be any guide as to its palatability for the fowl.

It is thus clear that the fowl has an acute sense of taste, which can profoundly influence its intake, making Engelmann's (1940) conclusion that chickens form food preferences on the basis of shape and colour but not by taste intrinsically unlikely. It is true that in virtually all the acceptability trials cited above the experimental subjects were presented with flavours in solution rather than in solid form, and it does seem probable that the fowl, because of its rather meagre supply of saliva, may be a good deal less sensitive to the taste of solid foods (Kare and Pick, 1960). However, Kare and Scott (1962) presented cockerel chicks

with a choice between a control diet of soya bean and maize meal and an experimental diet in which the maize was replaced by rye, barley or buckwheat. All three experimental diets appeared to be less palatable than the control, and the chicks selected the control diet, exhibiting a preference range of 74% - 91% in favour of it. The rye was the least palatable of the three experimental cereals, while the barley and buckwheat were very similar in acceptability. The selection could not be correlated with the nutritional adequacy of the diet since the barley diet gave as good, if not better, results as the maize when it was presented on its own. Both Graham (1932) and Banta (1932) observed that chickens exhibited definite preferences when allowed to select their diet from a selection of different foods, and these authors pointed out that both the flavour and the texture of the food appeared to be important in this selection. Kare and Pick (1960) have shown that the food intake of chickens can be greatly reduced by the addition of an offensive flavour to the diet.

It would thus appear that food preferences in fowls can be governed by shape, texture and flavour, and it seems probable that colour can also play a part. Hess (1956) reported that day old chicks exhibit preferential pecking at chips coloured orange, with a secondary peak in the blue. Yellow and green received a low score. However, it would appear that these innate preferences can be modified by experience, since in this department we have found that a mash which has been dyed green is selected in preference

to one dyed red or yellow, in a two choice situation. A possible explanation for this is that our chicks have been fed on a green coloured mash from day old to about six weeks, and thus have acquired a preference for green foods. It should also be pointed out that Capretta (1969) has found a very strong initial preference for green mash over red in naive chicks, which can be modified by comparatively brief experience. At first sight this seems to conflict with the results obtained by Hess (1956) but Capretta used a light green and a dark red dye, so there may also be an effect due to intensity differences.

Katz (1937) had found that newly hatched chicks would peck indiscriminately at almost any small discrete object which contrasted with its background. Food preferences will develop over time, and clearly experience will play a large role in this development. The same will be true of drinking behaviour. Hunt and Smith (1967) showed that initially pecks were directed preferentially at highly contrasting, shiny, spherical objects. If these stimuli were drops of water, a drinking reaction appeared to be triggered by the presence of water within the bill. They found that pools of water appeared to elicit no reaction at all from naive chicks, conflicting with the findings of Rheingold and Hess (1957), who had demonstrated that chicks would peck at stimuli mimicking the visual effects of water, particularly a bright reflective surface which could be set into motion. Indeed they had shown that mercury, a sheet of perspex and a pool of blue water were supernormal stimuli relative to plain water. It is

interesting that Hunt and Smith (1967) found that white spots on a black background were more attractive than black spots on a white background.

11. THEORIES OF MOTIVATION AND REINFORCEMENT AS THEY RELATE TO THE PROBLEM OF SPECIFIC APPETITES.

The point has now been made that organisms regulate the kinds of food they eat, and they do this to a certain extent according to the requirements of the body for carbohydrates, proteins, minerals and vitamins. This regulation is in general, imperfect, and can be influenced by factors other than the requirements of the particular dietary component in question. In addition, regulation appears to be much more efficient for some components than for others, and results which have been obtained in one species cannot necessarily be duplicated in others. With this in view, three general types of explanation have been offered to explain specific appetites.

The first proposes that specific appetite is innate. The animal has been provided with an inbuilt regulator of homeostasis, which maintains a steady state in the internal chemical environment of the organism. Changes which occur in the nutritional or metabolic needs of an animal act to modulate ingestion until the deficit is restored. The main proponent of this theory has been Richter, who proposed that an inborn specific appetite existed for protein, carbohydrate, fat and almost all minerals and vitamins (Richter, Holt and Barelare, 1938). He believed that in some cases,

e.g. sodium chloride, the sensory threshold of the receptors signalling the presence of salt was actually lowered, and that this was the mechanism leading to an increased intake of salt by sodium deprived animals. This theory does not stand up to close examination (Pfaffmann and Bare, 1950), but many later workers consider that there has indeed been some alteration in the animal's perceptual threshold, so that a deprived animal pays close attention to stimuli which the normal animal ignores (Bolles, Sulzbacher and Arant, 1964; Handal, 1965; Krieckhaus and Wolf, 1968), and that it ingests the appropriate nutrient under circumstances where no learning could possibly have occurred.

The second explanation proposes that specific appetite is learnt, but there are two distinct theories regarding the nature of what is actually learnt.

1. An animal associates some discriminable component of a nutrient or an adequate diet (taste, odour, texture or colour) with a sensation signifying 'well-being', that is the result of a post-ingestational feedback of signals evoked by repletion. This theory was first postulated by Harris et al. (1933) and later developed by Scott and his co-workers. The animal learns to identify and select those food items that lead to a beneficial effect after they have been ingested. This explanation has been used to account for the gradual time-course observed in the correctional phase of certain deficiencies. It also implies that an animal does not show a preference for an essential nutrient until a state of deficiency has actually developed, and there is indeed evidence for

this (Scott and Verney, 1949).

2. Rozin and his co-workers propose that when a specific appetite is seen what is actually being observed is a specific aversion to the deficient diet, rather than a specific preference for the adequate diet. This aversion is learnt, being related to the unpleasant and deleterious effect of the deficient diet. The evidence they produce is very persuasive, but is entirely based on the vitamin thiamine and its effects in the domestic rat, although Rodgers (1967) has extended the concept to other minerals. Nachman (1963b) has demonstrated that rats given one 10 minute trial drinking LiCl learned to avoid drinking it again, - indeed signs of aversion began to appear during the trial. The aversion generalised to NaCl and to a lesser extent to ammonium chloride, potassium chloride and water. After the aversion to NaCl had extinguished, the extinction generalised so that the rats would again drink LiCl. This result supports Rozin's hypothesis in that it indicates how aversive consequences can very rapidly convert an inbuilt preference for sodium chloride into a learned aversion.

The third explanation is in a sense an extension of the second, and its main advocate is Young (1967). The acceptance of nutrients is determined primarily by the quality and intensity of the emotional arousal (or palatability) evoked by oropharyngeal contact with them. Food items vary in their arousal value or palatability and can be ranked in terms of their acceptance by an individual or species. In this theory specific appetite is

just one of a large number of different factors which govern the ingestion of a particular food, habit strength being another. This explanation attempts to explain such puzzling facts as the consumption of greater quantities of nutrients than are required for growth or survival, the failure to ingest an essential nutrient when it is offered as a choice, or an addiction to sugar that interferes with the correction of a nutritional deficiency. The experiment by Harriman (1955), in which adrenalectomised rats with a preoperative preference for sugar failed to select salt in a 2 choice situation, is cited as support for the view that under some conditions a hedonic response can take priority over nutritional need. Young and Chaplin (1945) working with rats which had developed a preference for sugar over casein solution in their home cage, deprived them of protein and demonstrated that the sugar preference continued. When the rats were placed in a novel situation, however, they promptly exhibited a casein preference, yet the sugar preference was still present when they were returned to their familiar living cage. Young (1967) sees this as an expression of the enormous influence habit strength can have in the maintenance of a food preference. It should be pointed out however that Cullen and Scarborough (1969) repeated Harriman's (1955) experiment, but in a Skinner box rather than under ad libitum conditions, and found that rats, immediately they were adrenalectomised, switched to a preference for salt (1·5%), although preoperatively they had exhibited a preference

for sugar (13·5%). These authors fail to explain why they obtained such diametrically opposed results to Harriman, although they speculate that the necessity to make an operant response may disrupt previously learned habits in some way.

It is not easy to decide whether the apparent specific appetite observed in the case of amino acids warrants a fourth explanation or whether it can be subsumed under one of the above theories. When an animal deprived of an essential amino acid receives that amino acid into the cerebral circulation its food intake rises, whereas the same quantity of amino acid given into the general circulation has no effect upon food intake (Leung and Rogers, 1969). The authors ascribed this effect to the direct influence of the amino acid upon some central mechanism which controls voluntary food intake. However, rats show a preference for a complete diet over one identical except insofar that it lacks one essential amino acid (Sanahuja and Harper, 1963). In this two choice situation the animals show a typical learning curve, for their intake of the complete diet is initially at chance levels, but thereafter increases day by day. If the effect of the essential amino acid is simply that of increasing food intake it is impossible to explain the emergence of the differential preference, for it should stimulate consumption of the two diets equally. It is difficult to avoid the conclusion that one of the effects of the amino acid is to reinforce the intake of the enriched diet, and so that the effect is not an entirely automatic

one but that there are perceptual factors involved. It is not possible to determine from the experiments performed whether it is primarily a learned preference or a learned aversion.

Now that these different explanations have been presented, the conclusion is almost inescapable that learning must play a role in the development of specific appetites, with the possible exception of that for sodium chloride. There appears to be good evidence that this is truly innate, although even so learning may be involved under certain circumstances.

During the development of a specific appetite the animal must learn to associate some quality of the food with the effect it produces. The difficulty lies in the delay which may follow ingestion before the nutrient has any effect, for in general theories of learning demand a comparatively brief interval between a response and the reinforcement that follows it if learning is to occur (Hilgard, 1948). Recently, however, it has been demonstrated that learning with delays in reinforcement of the order of an hour or more can take place in rats. Garcia, Ervin and Koelling (1966) offered rats a saccharin solution for 10 minutes, and later apomorphine, which produces unpleasant internal effects, was administered. Following a few pairings of this nature, intake of saccharin decreased, even when an interval as long as 1 hour elapsed between saccharin ingestion and drug injection. Aversions have been produced to solutions whose consumption was followed by X irradiation, even when the interval was as long as

6 hours (Revusky, 1968) or 12 hours (Smith and Roll, 1967). These situations were all ones in which negative reinforcement was applied, but Garcia et al. (1967) have also examined the effect of positive reinforcement. Thiamine deficient rats were allowed to drink saccharin solution, and later were given an injection of thiamine, which caused their saccharin consumption to increase. Delayed injection had just as great an effect up to 30 minutes, there was still a substantial effect at 75 minutes, but no learning occurred at 180 minutes.

Rozin (1969) points out this kind of long-delay learning works almost exclusively in the taste modality, and that there is a strong tendency for taste conditioned stimuli to become attached to gastro-intestinal unconditioned stimuli. This might be attributed to evolutionary selection, in view of the close relationship of the taste modality to the feeding system.

Rozin demonstrates that aftertaste or regurgitation is not an important factor in bridging this delay by conditioning an aversion to one (but not the other) of two different concentrations of casein hydrolysate, using injection of apomorphine as the unconditioned stimulus. An aversion was readily learnt by the rats under these circumstances, whereas no aversion was seen when two identical concentrations were used, even though the aversive solution was linked with clear and obvious non-taste cues such as position and colour. Rozin (1969) concludes that at least part of the long-delay learning phenomenon is not explainable in terms of aftertaste and must be due to the central retention of a memory

trace. This long term storage is only applicable to the memory of the relevant taste, and is not applied to non-gustatory cues. It is postulated that the findings cited above reflect specialisation in the integrative mechanisms which serve these afferent modalities (Garcia et al., 1967). It is an adaptive development, for since the ultimate effects of food are delayed, effective gustatory-visceral associations need to span longer time intervals than do tele-receptor cutaneous associations '(sight/sound-touch).'

The type of learning involved in these findings incorporates both associative and discriminative components. Lat (1967) analyses critically a substantial number of experiments investigating the rat's ability to choose a satisfactory diet. He concluded that the factors which are common to those experiments which demonstrated successful self selection include:

1. Housing of animals in groups rather than individually.
2. Continuing the period of choice for 21 days or over.
3. Female rats were more successful than males.
4. Age and genetic constitution were also highly important.

Lat set out to answer the question of whether rats with a higher learning ability were also more successful in mastering self selection. He measured the speed of associative and discriminative learning with a Skinner box, and found that individuals with higher discriminative ability showed only a brief period of weight loss and then gained more weight under self selection conditions. Practically zero correlations appeared with the associative type of

learning. This suggests that discriminative rather than associative learning might be involved in the regulatory process.

12. EFFECT OF DEPRIVATION UPON ACTIVITY.

It is now generally accepted that one of the effects of food and water deprivation is to increase the amount of spontaneous activity seen in an animal (Baumeister, Hawkins and Cromwell, 1964). The increase in drive has the effect of alerting the animal, rather than actually activating it. Campbell and Sheffield (1953) measured the activity of rats in stabilimeter cages before and after starvation, and showed that although there was a small but significant increase in total activity, the rise was very much greater when an environmental change was introduced. They concluded that hunger drive involves a lowering of thresholds rather than having its effect as an internal drive stimulus. This increase in activity is to some extent a function of recording device, for food deprived rats exhibit only a slight increase in activity in a stationary cage, whereas the increase can be ten-fold in an activity wheel (Weasner, Finger and Reid, 1960). Considerable species differences may also be observed. Chicks deprived in a stabilimeter cage showed large increases in activity compared to satiated animals for 4 days, the activity falling back to basal levels at 7 days. Similar increases were seen in the case of both food and water deprivation. Rabbits, guinea pigs and hamsters showed no increase in activity in the stabilimeter, but guinea pigs and hamsters exhibited a rise in activity in

running wheels, though in the case of food deprivation only (Campbell, Smith, Misanin and Jaynes, 1966).

Some attention has been paid to the effect of deprivation of a specific nutrient rather than an overall food or water deprivation. Hitchcock (1928) found that a protein-free diet had the effect of increasing the activity of a rat for a short period of time, but that a low protein diet over a long period depressed activity. Richter and Rice (1943) fed rats on a diet consisting largely of maize, and showed that a deficiency of Vitamin D appeared to result in a gradual decrement in activity. Guerrant, Dutcher and Chornock (1939) reported a similar finding in the case of Vitamin A, deprived rats being less active than controls. The effect developed gradually over about one month. Wald and Jackson (1944) placed rats in a cage with a running wheel and observed that feeding a diet deficient in thaimine led to an increase in activity which continued until the neuromuscular symptoms appeared. Similar effects were noted with riboflavin, but they were not usually as prompt nor as marked as those associated with thiamine. On the other hand a magnesium deprivation was followed within a few days by a decline in activity to very low levels. It would seem, therefore, that depriving an animal of some of the essential components of its diet may have an effect similar to that produced by total food deprivation.

The experiments described above have all been concerned with the effects of food deprivation on so-called 'spontaneous'

activity - even though one of the main conclusions derived from them is that it has little effect on truly spontaneous activity, but tends to act by making the animals more sensitive to external stimuli. Thompson (1953) has examined the effect of food deprivation in a situation where activity might have a more adaptive function. Rats were placed in a novel maze after 24 or 48 hours of food deprivation. He found that exploratory behaviour was higher than that of controls after 24 hours deprivation in both male and female animals, whereas after 48 hours it was higher still in males, but actually lower in females than in the controls. This interesting result has not been followed up, and the sexual difference remains unexplained.

The mechanism governing these activity changes has not yet been elucidated, but Routtenberg and Kuznesof (1967) have put forward a hypothesis which relates bodily activity to hypothalamic function. Rats were maintained on a 23 hour food deprivation schedule, and showed maladaptive behaviour in that they indulged in a high level of wheel running activity and lost weight rapidly. Controls were maintained on the same schedule but did not have access to a wheel, ate more than the experimental animals and in general gained weight. It is postulated that food deprivation leads to hunger and weight loss, and thereby to overactivity in the lateral hypothalamus, which in turn increases locomotor activity. This activity generates metabolic products which react on hypothalamic centres to depress food intake. This finding can also be

accounted for in terms of arousal. There is evidence that these rats are in a condition of high arousal. Neither the arrival of food nor, initially, eating, would lower this state of high arousal, and therefore feeding responses would not be reinforcing, so the animal would tend to eat less. Although the activity appears maladaptive under these experimental conditions, it does not follow that this would be the case under more natural circumstances, where the increased activity could be integrated into more purposeful food-seeking behaviour.

INTRODUCTION TO THE EXPERIMENTAL WORK

AIMS AND APPROACHES OF THE PRESENT STUDY

In this work I investigated the existence of specific appetites in the domestic fowl. The findings of Wood-Gush and Kare (1966) with regard to the appetite for calcium seen in calcium-deprived birds were followed up and extended; the speed at which a preference formed was established; the role of learning as opposed to inborn, unlearnt tendencies in the development of the preference was investigated; and the degree of individual variation in groups of birds was determined. Other facets examined were the importance of cues such as colour, taste and texture, the effect of supplementing the diet with different calcium salts to confirm that the appetite was not limited to a particular salt, whether the appetite would generalise to other elements such as strontium, the differences seen when the calcium was provided in solution rather than in the food and the effect of injecting a calcium solution into the deprived birds. In view of Rozin's findings in rats, the effect of deprivation in producing a preference for novelty was also examined.

The role of reinforcement in the learning of a preference was explored by influencing a possible need-reduction mechanism using analgesic drugs, and also affecting changes in arousal by the use of tranquillisers and ethyl alcohol. I hoped to determine the value of calcium as a reinforcer in laying hens (which have a high requirement for this mineral) by seeing whether these birds would learn an operant response in a Skinner box to receive an

opportunity to ingest calcium grit.

The effect of deprivation on activity was investigated by observing the behaviour of normal and deprived chickens in an open arena, in particular their pecking preferences when they were presented with substances either containing calcium or deficient in it. Activity was also monitored in a stabilimeter cage, comparing the activity level of chickens as they became more deprived of calcium with that of controls receiving an adequate diet.

The possible role of hormones which govern the metabolic control of calcium absorption and excretion was examined by injecting a calcium mobilising hormone such as parathormone and observing its effect on behaviour.

In addition to these studies relating to calcium, a rather briefer examination of the effects of depriving birds of sodium and of thiamine was also carried out. It was hoped to deprive chickens of calcium by means of parathyroidectomy and of sodium by means of adrenalectomy, but for technical reasons these ablation experiments were not carried through, and deprivation was produced in both cases by means of feeding deficient diets. However, in the case of sodium, deficiency was also induced using the technique of subcutaneous formalin injection, which in rats depletes the circulating fluids of sodium, thus producing a functional deficiency. Thiamine deficiency was produced by dietary means, coupled with the injection of the thiamine antagonist, oxythiamine.

EXPERIMENTAL FINDINGS

EXPERIMENT 1.

THE INVESTIGATION OF THE EXISTENCE OF A PREFERENCE FOR CALCIUM CARBONATE AMONG CALCIUM DEFICIENT BIRDS.

Introduction.

This experiment was designed to compare the relative intake of two diets, one deficient in calcium (D. diet) and one containing a known proportion of calcium (Ca diet) over a period of time, by birds which were calcium deficient, in contrast to normal controls.

Subjects

These were thirty four broiler chickens of both sexes and 7 to 8 weeks old at the beginning of the period of deprivation. They were a well known commercial stock derived from New Hampshire and Columbian (female) and White Rock and Cornish Game (male).

Methods

The subjects were divided at random into two groups. The experimental group was fed a diet balanced in every respect except in its content of calcium which was only 0·02%. (see Diet 1 for details of composition). The control group was fed a similar diet but containing in addition approximately 1·5% calcium. They were given only distilled water to drink during this experiment, - though in later experiments birds received tap water since Edinburgh mains water contained approximately 20 ppm of calcium, so that the daily water intake would provide only some 4 mg. - a negligible quantity. At the end of 28 days of deprivation

representative birds from each group were bled from a wing vein and the plasma calcium levels were estimated using an atomic absorption spectrometer. At the conclusion of this period each bird was presented with two polystyrene boxes, each having a $2\frac{1}{2}$" hole in the lid. The purpose of these holes was to reduce spillage and waste of the diet. One box contained the D. diet in pelleted form, and the other the same diet with the addition of 2% precipitated calcium carbonate, giving a calcium level of 0·8%. This level should be an adequate one for birds of this age. The optimal level for maximal growth rate in broilers varies from 0·5% calcium (Simco and Stephenson, 1961), through 0·8% (Yoshida, Hoshii and Morimoto, 1967) to 1·05% (Edwards, Marion, Fuller and Driggers, 1963). The diets were similar but not identical in appearance, - the pellets of the Ca diet being somewhat greyer in colour. Each box contained about 165 gms. of food, the total weight of box and food being 200 gms. After 24 hours the amount of food eaten from each box was weighed, the weight was made up to 200 gms. again, and the position of the boxes was reversed from left to right and vice versa, in order to nullify the effects of a positional preference. In case there was a consistent bias towards either side, half of each group was first presented with the Ca diet on the left and half with it on the right. If the control birds showed no preference, eating an equal amount of both diets, their calcium intake would be only 0·4%. In order to obviate the possibility that at the end of ten days they too might be suffering from a calcium deficiency, albeit a slight one, they were force fed every 2 days with cockle

shell, being given sufficient to raise their daily calcium intake to approximately 1·5 gms. After 10 days the plasma calcium levels were again estimated and the experiment was discontinued. The experiment was performed in two parts - there being 8 experimental and 5 control birds in the first part and 11 experimental and 10 control birds in the second. The two parts were otherwise identical. The numbers of experimental and control birds are not the same owing to a greater mortality among the controls - including a case of sudden and unexplained death, two cases of accidental leg injury and one bird which had to be destroyed following bleeding.

The most difficult problem in assessing the results of this experiment was in deciding what exactly to define as a preference for calcium. The simplest answer would have been to have defined it as 'the consumption of more Ca diet than D diet over the 10 day period of choice'. This however would result in the discarding of a great deal of information, as one is not interested merely in the existence of a difference in consumption but also in the extent of that difference. This may be shown schematically in the following table (anticipating the results a little):

Ca diet eaten as a % of total	Normal Controls	Deprived Experimentals
0 - 10	11	1
10 - 20		
20 - 30	1	
30 - 40	1	
40 - 50	11111	11
50 - 60	1	1
60 - 70	1	111
70 - 80	11	11111
80 - 90	1	111
90 - 100	1	1111

Although one could easily take a higher figure such as 70% as indicating calcium preference, the actual figure taken would clearly be completely arbitrary, in view of the wide spread of the controls. The criterion finally settled upon, i.e. a greater consumption of Ca diet than of D diet on 7 out of the last 9 days, can be justified in the following terms. If the first 2 days are allowed as a settling in period the chicken then has to eat almost consistently more from the Ca diet for the remainder of the period. It can in fact reverse its preference on one day and still meet the criterion. This seems to be reasonable since one would not necessarily expect a preference to be established immediately (unless one subscribed to the specific hunger theory) but to emerge over a period of time. Evidence for this will be presented below.

Results

These are shown in Table 2.1 (and in detail in Table 2.1A in the Appendix). On the basis of their feeding behaviour the subjects were classified into four groups. These groups were - calcium preference, calcium rejection, positional preference and no preference. The results from the first day were discarded, as it appeared that these were greatly influenced by whichever box a bird happened to start eating from first. (Nine birds ate more over the first day from the box they first sampled, two ate less, and two ate much the same from both). The classification dealt therefore with the final nine days, and the criterion for calcium preference was that the bird must score 7 out of 9. One

point was given for each day on which it ate more of the Ca diet than of the D diet. If equal amounts were eaten from each box it was given half a point. Ideally these 7 or more days should be consecutive - that is, once a preference has been exhibited it should be maintained - but the birds have to decide which of two variables (i.e. position or diet) is the important one, and so a gap of one day in the sequence is permitted and this was shown by two birds. B 4340 had a sequence of 7 days with two gaps of one day, but on these two days an almost equal amount of both diets was consumed, so it was placed in the calcium preference group. B 4968 showed four days of positional preference, abruptly followed by 5 days of very marked calcium preference (showing learning of the "eureka" type ?). The experiment was therefore continued with this bird for a further three days after Day 10 and during this period it continued to show calcium preference so was placed in this group.

Only one experimental bird was placed into the calcium rejection group, and it was noted that throughout the 10 day period this bird had never eaten more than 5 gms. of the Ca diet on any one day and only a total of 12 gms. in all (compared with 771 gms. of the D diet.). It was postulated that it could not have experienced any beneficial effect from such a small amount (should this beneficial effect exist) and so at the conclusion of the 10 day period it was presented with the Ca diet in both boxes, so that it had no choice but to eat it. After 24 hours it was given a choice again as before - the D diet being placed in the box from

which it had eaten most, in order to nullify any cue effect from the box, and each day the diets were switched from box to box as well as from side to side. The results were as follows:

Bird No.	Day	11	12	13	14	15	16	17
B 4702	Ca diet	106	87	88	102	102	125	93
	D diet	-	0	0	0	0	0	0

The bird now shows a complete preference for calcium, in utter contrast to its previous rejection. This result is of course only suggestive but nevertheless most interesting.

A minor problem was the spillage of food. Some birds spilt more than others but even in the worst cases it did not amount to more than about 1·5%. It was not subtracted from the total - partly because of the difficulty of sorting out the pellets accurately into their two groups, and partly because in order to spill it a bird had to lift a pellet clear of the box before releasing it, and thus in a sense it had selected a pellet, although it did not in fact eat it.

The results are summarised in Table 1 - and are given separately for the two batches and then combined. They were examined for statistical significance using the method of Freeman and Halton (1951). This involves calculating the probability of occurrence of all the possible distributions which are as extreme (and also which are more extreme) than the distribution actually obtained. The actual calculation was carried out by the KDF 9 computer for which a program called "Conprob" was written.

Table 1

Statistical Evaluation of the Results

Batch	Group	Ca pref.	Ca Rej.	Posit. Pref.	No. Pref.	Probability
1	Exp.	6	0	2	0	
	Cont.	2	1	0	2	P = 0·0754
2	Exp.	9	1	0	1	
	Cont.	3	2	2	3	P = 0·0827
1 + 2	Exp.	15	1	2	1	
	Cont.	5	3	2	5	P = 0·0251

From Table 1 it may be observed that in the case of both Batch 1 and Batch 2 the calcium deprived birds show a greater preference for calcium carbonate than the controls, but that this preference is not significant. However, when the results for the two batches are combined the preference is a significant one at the 5% level (P = 0·025).

In Table 2.1 the relative proportion of the Ca diet eaten by each bird is expressed as a percentage. There is a fairly close agreement between the criterion of 7 out of 9 and a total intake over the 10 days of 75% Ca diet. The results are also expressed graphically in terms of the percentage of Ca diet eaten by the entire batch each day, in order to give some idea of the daily trend in consumption (Fig. 1). From this figure it can be seen that on the first day both groups average about 50%, and that subsequently the intake of the controls remains around this

chance level while that of the deprived birds climbs fairly steadily until it settles down at about 80%.

Table 2.2 (and 2.2A in the Appendix) show that the plasma calcium levels of the deprived birds are very much lower than those of the controls at the beginning of the choice period, whereas they have risen substantially and are not significantly different by the end of the period.

Conclusions

This experiment confirms the existence of a preference for calcium among calcium deprived chickens. It also suggests that the preference is not an unlearned specific hunger in the sense that it is immediately present at its maximum strength, but that on the contrary it takes a period of time, probably measured in days under the conditions of this experiment to build up.

Table 2.1

Batch 1

Experimental (Calcium deprived) birds

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca No.	Ca %
B 4345	C	C	C	C	C	C	D	C	C	C	8	69·9
B 4340	C	C	C	D	C	C	C	D	C	C	7	59·9
B 4339	D	C	C	C	C	C	C	C	C	C	9	83·8
B 4350	C	D	C	D	C	D	C	D	C	C	5	62·3
B 4336	D	C	C/D	C	C	C	C	C	C	C	8½	66·8
B 4337	C	C	C	C	C	C	C	C	C	C	9	76·8
B 4349	D	C	D	C	D	C	D	C	D	C	5	49·1
B 4344	C	D	C	C	C	C	C	C	C	C	8	78·5
Ca %	46	67	62	64	68	73	72	78	80	80		

[Contd.

Table 2.1 (Contd.)

Batch 1 (contd.)

Normal Control birds

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca No.	Ca %
B 4343	C	C	C	C	D	D	D	C	-	-	(4)	57·4
B 4338	D	D	D	C	C	C	C/D	C	C	C	6½	41·2
B 4342	C	C	C	C	C	C	C	D	D	D	6	74·7
B 4348	C	D	C	C	C	C	C	C	C	C	8	78·3
B 4347	D	D	D	D	D	D	D	D	D	D	0	2·3
Ca %	45	50	53	68	62	60	58	53	53	52		

Batch 2

Experimental birds

B 4968	D	C	D	C	D	C	C	C	C	C	7	76·4
B 4707	D	C	C	C	C	C	C	C	C	C	9	92·0
B 4695	D	C	C	C	C	C	C	C	C	C	9	71·9
B 4702	D	D	D	D	D	D	D	D	D	D	0	1·5
B 4699	D	D	C	C	C	C	D	C	D	D	5	47·0
B 4579	C	C	C	C	C	C	C	C	C	C	9	98·0
B 4585	C	C	C	C	C	C	C	C	C	C	9	99·1
B 4704	C	C	C	C	C	C	C	C	C	C	9	88·7
B 4581	D	C	C	C	C	C	C	C	C	C	9	92·1
B 4583	C	C	C	C	C	C	C	C	C	C	9	86·3
B 4696	D	C	D	C	C	C	C	C	C	C	8	76·4
Ca %	43	73	66	85	78	92	81	87	81	80		

Control birds

B 4967	D	D	D	D	D	D	D	D	D	D	0	6·1
B 4580	C	D	D	D	C	C	D	C	D	D	3	40·5
B 4584	C	D	C	C	C	C	C	C	C	C	8	97·0
B 4577	D	D	D	C	D	C	C	D	D	C	4	48·7
B 4700	D	D	D	C	C	C	C	C	C	D	6	66·4
B 4701	C	D	C	C	C	C	C	C	C	D	7	86·1
B 4709	D	C	D	C	D	C	D	C	D	C	5	42·9

[Contd.

Table 2.1 (Contd.)

Batch 2 (Contd.)

Control birds (contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca No.	Ca %
B 4708	D	D	C	D	D	D	D	D	D	D	1	29·0
B 4705	D	C	D	C	D	D	D	D	C	C	4	48·3
B 4782	D	D	D	C	D	C	D	C	D	C	4	39·1
Ca %	49	46	37	58	48	60	45	58	49	50		

Combined totals of Batches 1 and 2:

Experimental birds

Ca %	45	70	64	77	74	83	77	83	80	80

Control birds

Ca %	47	48	43	61	53	60	49	51	50	51

Note: C indicates that the bird ate more of the Ca diet.

D indicates that the bird ate more of the deficient diet.

C/D indicates that it ate equal amounts of both diets.

Table 2.2

Blood Calcium Levels

Group	Ca level before choice	Ca level after choice
Deprived	9·1 (± 0·67) mg/100 mls	12·6 (± 0·21) mg/100 mls
Control	17·1 (± 0·53)	13·0 (± 0·54)

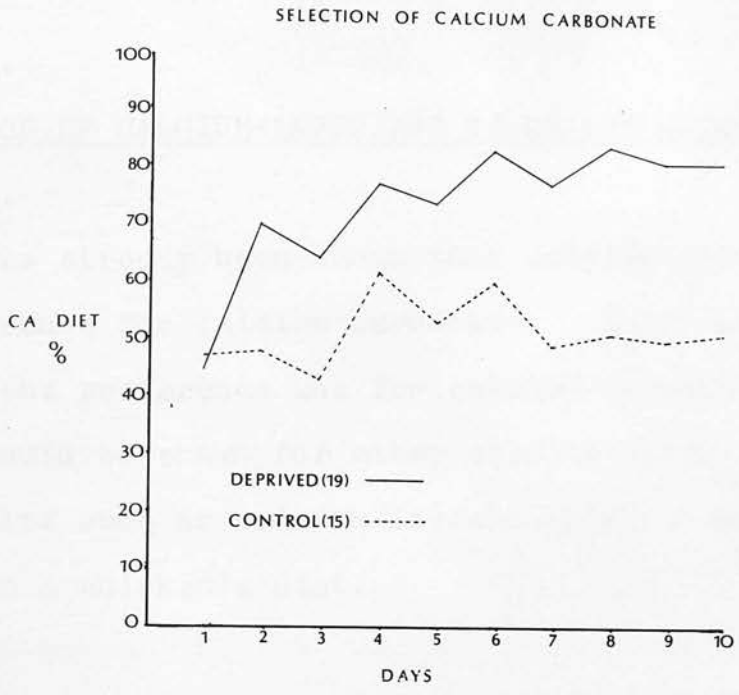


Fig. 1. Choice between a calcium-deficient diet and the same diet with 2% calcium carbonate.

(Experiment 1)

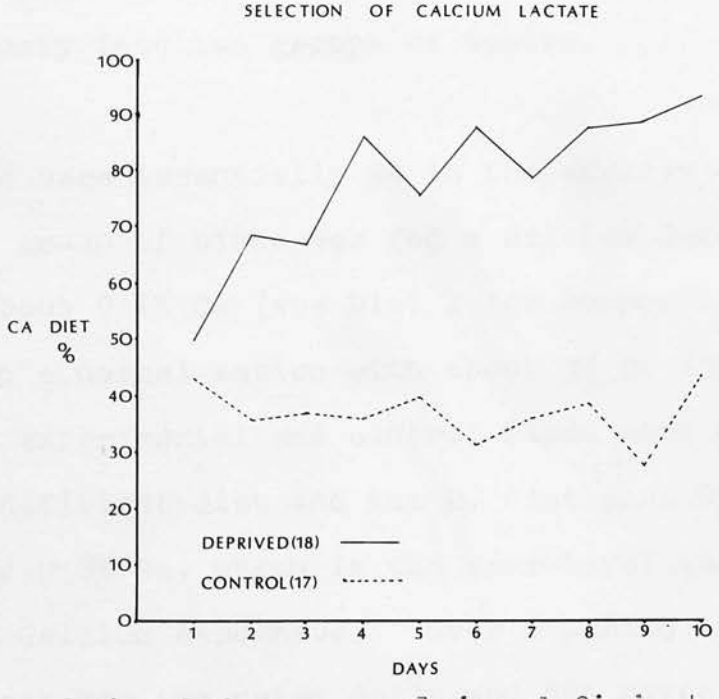


Fig. 2. Choice between a calcium-deficient diet and the same diet with 6% calcium lactate.

(Experiment 2)

EXPERIMENT 2.

THE PREFERENCE OF CALCIUM-DEFICIENT BIRDS FOR CALCIUM LACTATE.

Introduction.

It has already been shown that calcium-deprived chickens show a preference for calcium carbonate. This experiment was to see whether the preference was for calcium carbonate as such, or whether it would be shown for other calcium salts - especially synthetic salts such as calcium lactate which would not normally be present in a chicken's diet.

Subjects

The experiment was done in two stages. In the first, 11 six-week old broilers were divided at random into groups of six and five, and in the second 24 seven-week-old broilers were divided randomly into two groups of twelve.

Methods

These were essentially as in the earlier experiment. The experimental group of birds was fed a calcium deficient mash - containing about 0·1% Ca (see Diet 1 for composition) and the control group a normal ration with about 3% Ca (Diet 5). After 28 days both experimental and control birds were offered a choice between the deficient diet and the D. diet plus 6% calcium lactate. This contains 0·8% Ca, which is the same level as the earlier diet with 2% Calcium carbonate. Over a period of 10 days the amount eaten from each box was noted daily and the boxes were changed around from left to right.

Results

These are shown in Table 3, and in full detail in 3A in the Appendix, and are summarised below:

Batch 1

Group	Ca preference	Ca rejection	No pref.	Posit. pref.
Deprived birds	4	-	1	1
Normal controls	-	5	-	-

Two of the deprived birds were rather difficult to classify. One seemed to have switched over to a calcium preference in the last three days after showing a positional preference, - while the other showed six days of calcium rejection followed by three days of calcium preference. Each bird was followed through for a further four days to see whether the calcium preference was indeed established.

		July 24	25	26	27
B 7043	Ca	78	116	91	93
		45	6	13	1
B 7044	Ca	101	118	125	115
		24	34	30	22

It does appear that the calcium preference has become established, - however since these two birds did not, strictly speaking, meet the criterion (7 Ca choices in the last 9 days of the experiment) they were classified as 'no preference' and 'positional preference' respectively.

Batch 2

Group	Ca pref.	Ca reject.	No pref.	Posit. pref.
Ca deprived	12	-	-	-
Normal contr.	-	5	6	1

When batches 1 and 2 are combined the overall results are:

Group	Ca pref.	Ca reject.	No pref.	Posit. pref.
Ca deprived	16	-	1	1
Normal contr.	-	10	6	1

The probabilities of these distributions occurring by chance were calculated using the computer program 'Conprob' and were as follows:

Batch 1 P = ·006

Batch 2 P = ·000001

Combined P = ·0000001

Conclusions

From the results it is quite clear that calcium deprived birds show a very marked preference for calcium lactate - even more so than for calcium carbonate - although they have had no previous experience of it. It would also appear that normal birds find it somewhat distasteful - the proportion of those which rejected it being much higher than in the case of calcium carbonate (10 out of 17 as opposed to 3 out of 15). When the results are expressed on a massed basis in graphical form the differences appear very dramatic (Fig. 2). This experiment provides support for the hypothesis that there is no built-in preference for any particular calcium salt, but that it is calcium as such which is selected, and that this preference is built up by a learning process, which under the particular conditions of this experiment takes days rather than hours. A probable reason for birds showing a more marked preference for calcium lactate than

for carbonate is the stronger taste of the lactate acting as a more obvious cue, and allowing the bird to identify the two diets with more certainty.

Table 3

Batch 1.

Deprived

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
B 7045	D	D	C	C	C	C	C	C	C	C	85·0	8
B 7054	D	C	D	C	C	C	D	C	C	C	60·6	7
B 7047	D	C	C	C	C	C	C	C	C	C	82·4	9
B 7044	D	C	D	C	D	C	D	C	C	C	58·6	6
B 7043	C	D	D	D	D	D	D	C	C	C	42·1	3
B 7050	C	D	C	C	C	C	C	C	C	C	75·2	8
Ca %	36	52	51	79	61	82	66	76	81	89		

Control

B 7049	D	D	D	D	D	D	D	D	D	D	37·4	0
B 7046	D	D	D	D	C	D	D	D	D	C	35·4	2
B 7052	D	D	D	D	D	D	D	D	D	D	22·0	0
B 7051	D	D	D	D	D	D	D	D	D	C	22·8	1
B 7048	D	D	D	D	D	D	D	D	D	D	9·6	0
Ca %	39	22	29	22	25	20	21	23	19	49		

Batch 2

Deprived

B 7256	C	C	C	C	C	C	C	C	C	C	99·1	9
B 7258	D	C	D	C	D	C	C	C	C	C	78·1	7
B 7259	D	C	D	C	C	C	C	C	C	C	65·4	8
B 7260	C	C	C	C	C	C	C	C	C	C	94·3	9
B 7261	C	C	C	C	C	C	C	C	C	C	92·7	9
B 7263	D	C	C	C	C	C	C	C	C	C	90·0	9

[Contd.

Table 3 (Contd.)

Batch 2 (contd.)

Deprived (contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
B 7264	C	C	C	C	C	C	C	C	C	C	94·0	9
B 7265	C	C	C	C	C	C	C	C	C	C	71·7	9
B 7267	D	C	C	C	C	C	C	C	C	C	83·7	9
B 7282	D	C	C	C	C	C	C	C	C	C	75·7	9
B 7284	C	C	C	C	C	C	C	C	C	C	93·8	9
B 7311	C	C	C	C	D	C	C	C	C	C	77·0	8
Ca %	58	78	75	90	84	91	85	95	95	96		
Control												
B 7273	D	D	D	C	C	C	C	C	D	C	52·4	6
B 7274	C	D	D	D	D	D	D	D	D	D	40·8	0
B 7276	D	C	D	C	D	D	D	C	D	D	46·1	3
B 7278	D	D	D	D	D	D	D	D	D	D	19·8	0
B 7283	C	D	D	D	D	D	D	C	D	D	31·5	1
B 7285	C	C	D	D	C	C	D	C	D	C	49·5	5
B 7309	C	C	D	D	C	D	C	D	D	C	46·1	4
B 7313	C	D	C	D	C	D	C	D	D	D	46·3	3
B 7316	D	D	D	D	D	D	D	D	D	D	31·8	0
B 7318	D	D	D	D	D	D	D	C	D	D	21·4	1
B 7320	C	C	C	C	C	C	D	D	D	D	60·6	5
B 7322	C/D	D	C	C	C	D	D	D	D	D	48·5	3
Ca %	45	43	41	42	46	36	42	44	34	41		
Batches 1 and 2												
Deprived												
Ca %	49	69	67	86	75	88	79	88	89	93		
Controls												
Ca %	43	36	37	36	39	31	35	38	27	43		

C indicates that more of the Ca diet was eaten

D indicates that more of the deficient diet was eaten

C/D indicates that equal amounts of both were eaten.

EXPERIMENT 3.1
SELECTION OF CALCIUM-CARBONATE-ENRICHED MASH WHICH HAS BEEN ADULTERATED WITH QUININE

Introduction

In Experiment 2 the suggestion was made that the presence of a strong though unpleasant flavour actually assisted calcium deprived chickens in making an appropriate choice. This experiment was intended to test this suggestion using quinine to impart a taste to the diet, since the flavour of quinine is known to be aversive to chickens at a very low concentration.

Subjects

18 eight-week-old broilers of both sexes.

Materials and Methods

The subjects were divided at random into a group of 10 and a group of 8 and these were then split into halves. The 10 birds were fed the calcium-deficient diet (Diet 2) for 6 weeks while the 8 controls received the breeders ration (Diet 5) containing 3% of calcium. At the end of this period each bird was given a choice between two diets which were presented to it in plastic boxes, as described in Experiment 1. 5 deprived birds and 4 controls were given a choice between the low calcium diet and the same diet to which had been added 2% calcium carbonate powder and 0·2% quinine hydrochloride. The other 5 deprived and 4 control birds were given a choice between low-calcium diet and the diet supplemented with 2% calcium carbonate only. Intake from these boxes was recorded daily for a period of 10 days, the

position of the boxes being reversed from left to right each day.

Results

The results are shown in Table 4 and in full detail in Table 4A in the Appendix. A summary is given below, in which the different groups have been compared with each other. For example, in the first section, the deprived birds which have received the choice in which the calcium supplemented diet has been adulterated with quinine are compared with those who have been offered unadulterated calcium diet. In the second section the controls are similarly compared, and so on. These results were examined for significance using the method of contingency called 'Conprob' described in Experiment 1.

Group	Ca Pref.	Ca Rej.	Pos. Pref.	No Pref.	Probability
Deprived					
Quinine	0	5	0	0	P = ·008
Unadulterated	4	0	0	1	
Control					
Quinine	0	4	0	1	P = ·028
Unadulterated	2	0	0	2	
Quinine					
Deprived	0	5	0	0	P = 1·00
Control	0	4	0	0	
Unadulterated					
Deprived	4	0	0	1	P = ·524
Control	2	0	0	2	

Discussion

It is immediately apparent that these birds rejected the calcium diet adulterated with quinine, whether they were calcium-deprived or not. When both choices were unadulterated the deprived group showed a rather greater preference for the calcium-supplemented diet, but so did the controls and the difference was not significant. It is quite clear that the addition of quinine, at this level, far from acting as a taste cue, actually blocks completely the preference for the calcium-supplemented diet which would normally be seen in the deprived birds.

Table 4

Deprived (Quinine choice)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
D 423	D	D	D	D	D	D	D	D	D	D	11·0	0
D 350	C	D	D	D	D	D	D	D	D	D	8·9	0
P 1427	D	D	D	D	D	D	D	D	D	D	8·9	0
P 1415	D	D	D	D	D	D	D	D	D	D	21·4	0
P 1413	D	D	D	C	D	C	D	D	D	D	21·6	
Ca %	38	14	15	15	15	13	10	11	9	9		
P 1453	C	D	D	D	D	D	D	D	D	D	9·9	0
D 418	D	D	D	D	D	D	D	D	D	D	10·0	0
D 420	D	D	D	D	D	D	D	D	D	D	28·1	0
D 417	D	D	D	D	D	D	D	D	D	D	5·5	0
Ca %	42	26	20	18	12	9	7	3	1	5		

[Contd.

Table 4 (Contd.)

Deprived (Unadulterated choice)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
P 1440	D	C	D	C	C	C	C	C	D	C	58·3	7
P 1418	D	C	C	D	C	D	C	C	C	C	52·8	7
D 413	C	C	D	C	C	D	C	C	C	C	58·4	7
P 1409	C	D	C	C	C	C	C	D	C	C	55·4	7
C 8706	C/D	D	C	D	C	D	C	C	C	C	51·7	6
Ca %	50	45	50	54	62	47	64	60	54	64		

Controls (Unadulterated choice)

C 8101	D	D	D	D	C	D	D	C	D	C	42·9	3
P 1422	D	C	C	C	C	C	C	C	C	C	59·5	9
C 8703	C	D	C	C	C	C	C	D	C	C	57·4	7
P 1451	D	C/D	D	C	C	C	C	C	D	C	54·6	6½
Ca %	45	46	51	58	62	58	52	57	51	57		

EXPERIMENT 3.2

ADULTERATION WITH QUININE AT 0·1%

Introduction

This experiment is a replicate of 3.1 except that the calcium-supplemented diet is being adulterated with quinine hydrochloride at a level of 0·1% rather than at 0·2%, in view of the extreme aversiveness of this latter level.

Subjects

20 eight-week-old broilers of both sexes.

Materials and Methods

Exactly as in the previous experiment except that there

were 5 birds in each of the four groups, and that quinine hydrochloride was being added at a level of 0·1%.

Results

As before the results are summarised and the probability that any differences between them have occurred by chance is calculated.

Group	Ca Pref.	Ca Rej.	Pos. Pref.	No Pref.	Probability
Deprived					
Quinine	0	3	2	0	P = ·008
Unadulterated	5	0	0	0	
Control					
Quinine	0	4	0	1	P = ·029
Unadulterated	2	0	1	2	
Quinine					
Deprived	0	3	2	0	P = ·444
Control	0	4	0	1	
Unadulterated					
Deprived	5	0	0	0	P = ·167
Control	2	0	1	2	

Discussion

The results obtained in this replicate are essentially the same as in 3.1. There was again a significant difference between the group offered the unadulterated calcium diet or that offered the same diet adulterated with quinine, and this was the case with both deprived and control birds. However, the quinine diet is not as aversive - the birds ingested more than they had done at the 0·2% level, and there is a slight difference between deprived and control

birds, though it is not nearly significant. When the results for the 2 replicates are added together the difference between the deprived and the control groups offered the unadulterated diet almost reaches significance (P = ·071). Clearly, the control group is finding this calcium supplemented diet more palatable than in some of the earlier experiments.

Conclusion

Two points emerge from this experiment. Strong though the preference for calcium may appear in most deprived birds, it can still be blocked by the addition of a very small amount of a highly aversive substance such as quinine. One reason for this may be that the chickens show an immediate and marked aversion for the calcium diet and thus have no opportunity to experience any possible beneficial effect of the calcium. It is possible that a different result might be seen if quinine were added to a diet for which a powerful preference already existed. The other point is that quinine does not act as a taste cue at this level and where there is already sufficient difference between the two diets. It would be difficult to show any discernible improvement over the performance of the 'unadulterated, deprived' group in 3.2.

Table 5

Deprived (Quinine choice)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
D 1029	D	D	D	D	D	C	D	D	D	D	21·4	1
D 1041	D	C	D	D	D	D	D	D	D	D	22·2	1

(Contd.

Table 5 (Contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
Deprived (Contd.)												
D 1022	D	C	D	C	D	C	D	C	D	C	57·8	5
D 1025	D	C	D	C	D	C	D	C	D	C	59·0	5
D 1038	D	C	D	C	D	D	D	D	D	D	34·0	2
Ca %	24	75	12	75	20	66	10	55	14	59		
Controls (Quinine choice)												
D 1011	D	D	D	D	D	D	D	D	D	D	20·6	0
D 1017	D	C	D	C	D	D	D	D	D	D	29·0	2
D 1016	D	D	D	D	D	D	D	C	D	D	32·5	1
D 1021	D	C	D	C	D	C	D	D	D	C	34·2	4
P 1441	C	C	D	D	C	D	D	D	D	D	46·2	2
Ca %	29	58	21	37	35	33	26	38	24	39		
Deprived (Unadulterated choice)												
D 1024	C	C	C	C	D	C	D	C	C	C	73·5	7
P 1429	D	C	C	C	C	C	C	C	C	C	84·4	9
D 1033	D	D	D	C	C	C	C	C	C	C	73·9	7
D 1019	C	C	C	C	C	C	C	C	C	C	93·7	9
D 1036	D	C	C	C	C	C	C	C	C	C	89·0	9
Ca %	49	77	86	95	76	89	78	96	85	98		
Controls (Unadulterated choice)												
D 1027	C	D	C	D	C	C	C	C	C	C	69·1	7
D 1026	C	D	C	D	D	D	C	D	C	C	52·2	4
P 1439	D	C	D	C	D	C	D	C	D	C	52·8	5
D 1018	D	D	C	C	C	C	C	C	C	C	79·6	8
P 1412	C	D	C	C	C	D	C	D	C	D	56·2	5
Ca %	49	52	61	51	59	69	61	65	78	70		

EXPERIMENT 4.1.

THE SELECTION OF CALCIUM-CARBONATE-ENRICHED MASH BY CALCIUM-DEFICIENT CHICKENS.

Introduction

This experiment was intended to supplement the results obtained in Experiment 1 so that numbers would be sufficiently large for statistically significant results to be obtained. In the earlier experiment the diets had been fed in the form of pellets, but in the meantime the pelleting machine had broken down so it was decided now to feed them in the form of mash.

Subjects.

These 15 eight-week-old broiler chickens were divided at random into an experimental group of 6, and a control group of 9.

Methods

The experimental group were fed a ration (see Diet 1) containing 0·03% calcium while the controls were fed a breeders ration (Diet 5) with an adequate amount of calcium (approximately 3%). After 28 days both groups were offered a choice between the deficient diet alone and the same basal diet with the addition of 2·0% powdered calcium carbonate. The procedure was exactly as in Experiment 1.

Results

These are given in Table 6 in full detail in Table 6A in the Appendix and in summary below.

Group	Calc. Pref.	Calc. Rej.	No Pref.	Posit. Pref.
Calc. deprived	-	1	4	1
Norm. controls	1	4	4	-

This distribution could very probably have occurred by chance. Using the computer program 'Conprob' it was calculated that P = 0·524. Of the four deprived birds which showed no preference only one, B 4937 came close to reaching the criterion (7) with a score of $6\frac{1}{2}$ over the last nine days of choice. It also showed the highest percentage calcium intake in either group (64·2%). The total daily intake of calcium diet (Table 6) shows a much greater separation between deprived and control groups over the last three days of choice, but the total number of birds was small, and this type of fluctuation can readily occur by chance.

Conclusions

In an earlier experiment with precisely the same diets as here the deprived group showed a significant preference for the calcium diet, so clearly an attempt must be made to account for the fact that they did not in this case. The only respect in which these diets differ from the earlier ones where the birds had discriminated concerns the texture - the earlier diet had been pelleted whereas this one was in the form of mash. The two forms of the pelleted diet were easily distinguishable, as the enriched diet was rather greyer owing to its higher calcium carbonate content. In the form of mash however the two diets were indistinguishable to the human eye, because of the high casein content both appearing

very dusty. It is suggested that the birds were unable to identify the diets, in spite of the fact that one diet contained 2% calcium carbonate and so one might have expected some difference in flavour, and therefore when they experienced a beneficial effect following the ingestion of a substantial amount of the enriched diet this did not reinforce increased consumption in the future. A positional effect might have been postulated - e.g. if a large amount of the enriched diet was eaten one day while it was on the left-hand side, the following day an increased consumption of the deficient diet (which was now on the left-hand side) might be expected. However this pattern was only noted in one of the birds (B 4936).

Table 6

Deprived

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
B 4928	D	D	D	D	D	D	D	C	D	C	40·1	2
B 4931	C	D	C	D	C	D	C	C	C	D	51·3	5
B 4936	D	C	D	C	D	C	D	C	C	D	48·8	5
B 4937	C	C	D	C/D	C	C	C	C	D	C	64·2	6½
B 5050	C	C	D	C	D	D	D	C	C	C	55·6	5
B 5051	D	D	D	C	D	D	D	C	C	C	47·1	4
Ca %	54	45	44	46	47	49	49	67	53	61		
Controls												
B 4929	C	D	C	C	C	D	C	D	D	D	51·6	4
B 4933	D	D	D	D	D	D	C	D	C	D	40·9	2
B 4934	D	C	C	C	D	C	D	D	D	C	49·5	5

[Contd.

Table 6 (Contd.)

Controls (Contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
B 4935	D	C	D	C	C/D	D	D	D	D	D	49·3	2½
B 5042	D	C	C	C	D	C	D	C	C	D	51·3	6
B 5045	D	C	C	C	C	D	C	C	C	C	52·2	8
B 5046	C	D	D	D	D	D	C/D	D	D	D	42·6	½
B 5054	C	D	C	D	D	D	D	C	D	D	50·1	2
B 5057	C	C	D	D	D	D	C	D	D	D	43·3	2
Ca %	41	52	56	51	48	47	50	44	45	43		

EXPERIMENT 4.2.

THE SELECTION BY CALCIUM-DEPRIVED CHICKENS OF TWO VISUALLY SIMILAR DIETS, ONE OF WHICH CONTAINS CALCIUM CARBONATE.

Introduction

This experiment was expected to throw more light on the results of Experiment 4.1 - in which birds had failed to discriminate a calcium-enriched diet from a deficient diet. These earlier diets were identical in appearance, and so it was tentatively concluded that visual cues were very important in discrimination. In this present experiment an attempt was made to formulate a pair of diets, calcium-deficient and supplemented, which would be visually identical but which would in all probability differ slightly in flavour.

Subjects

21 eight-week-old broiler chickens of both sexes.

Methods

These birds were divided at random into two groups. The deprived group were fed a diet adequate in all respects other than in its calcium content, which was only 0·03% (Diet 2). The control group was fed a breeders ration containing approximately 3% calcium (Diet 5). After 30 days both groups were placed in single cages and offered a choice between the calcium deficient diet and the same diet with the addition of 2% calcium carbonate. The addition of the calcium carbonate made that diet whiter and more dusty in appearance, so a certain amount of white flour was added to the deficient diet until the two were visually matched. The experimental procedure of daily weighing and reversal of diets was as described in earlier experiments.

Results

These are given in Table 7, and in full detail in 7A, and in summarised form in the table below.

Group	Ca Pref.	Ca Reject.	No Pref.	Posit. Pref.
Calc. deprived	8	-	3	-
Norm. controls	1	1	8	-

The significance of these results was assessed using the computer program 'Conprob' and the probability that this distribution could have occurred by chance is less than ·01 (P = ·007). Clearly the deprived birds show a very strong preference for the calcium-supplemented diet in comparison with the controls.

Conclusions

It would seem therefore from these results that chickens

do not require visual cues when establishing a preference for calcium carbonate,and to this extent the results are in direct conflict with those of the earlier experiment where the chickens failed to discriminate between the two diets. However, a number of points should be made. Although the results as shown in the summarised table are clear cut enough, when the detailed table is examined it will be seen that the calcium preference exhibited by the deprived birds is not as extreme as in some of the other earlier experiments. For example, bird B 7790 met the criterion in that it selected the Ca diet on 7 out of the last nine days, and yet over the entire period only ate 58·4% of its total intake from the Ca diet, so the bias in favour of the Ca diet was not very great. This is brought out even more clearly by the group figures for Ca diet %, when, after high figures for the first five days the percentage drops below 60% for the next four (see Fig. 3). It is true that these figures include those for three birds which did not show a calcium preference, but still in most previous experiments the figures have shown a rising trend over this part of the period. It is possible therefore that although a preference is shown the birds have greater difficulty in establishing and maintaining it than when visual cues are present.

There is an important difference between this experiment and Experiment 4.1 where no preference could be detected - the composition of the diets. Previously the diet was as shown in Diet 1, i.e. composed largely of a wheat-maize mixture with a

protein supplement of lactic casein. This 13% of casein made the diet very white and dusty when in mash form. The present diet had the composition shown in Diet 2 - i.e. mainly maize, with a protein supplement of soya bean meal, and it is probable that the addition of 2% calcium carbonate added the characteristic chalky flavour to the diet, whereas in the case of the casein diet any change in flavour was completely masked by the casein, which at least to man has a very strong and rather dusty flavour.

These conclusions are only tentative at this stage but may be summarised as follows:

1. Calcium deficient chickens have a preference for a calcium enriched diet.
2. This preference can only be shown if the diet is distinctive in colour (Experiment 1) or in taste (Experiments 2 and 4.2).
3. If the taste and colour of both diets are identical, no preference is exhibited (Experiment 4.1).

Table 7

Deprived

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
B 7790	C	C	C	C	C	C	C	D	C	D	58·4	7
B 7430	C	C	C	C	C	C	C	C	C	C	71·8	9
B 7849	D	C	C	C	C	C	C	C	C	C	73·3	9
B 7853	C	C	C	C	C	D	C	D	D	C	55·7	6
B 7789	C	C	C	C	C	C/D	C	C	C	C	75·5	8½
B 7857	D	C	D	C	C	D	C	C	D	C	55·0	6
B 7853	C	C	C	C	C	C	C	D	C/D	C	66·1	7

[Contd.

Table 7 (Contd.)

Deprived (Contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
B 7858	C	C	C	C	C	C	D	C	C	C	83·8	8
B 7788	C	C	C	D	C	C	D	D	D	D	56·6	4
B 7784	C	C	C	C	C	C	C	C	C	C	82·6	9
B 7783	C	C	C	C	C	C	C	C	C	C	76·9	9
Ca %	68	76	76	81	79	60	58	58	57	71		
Controls												
B 7789	C	C	C	C	C	C	C	D	D	D	60·8	6
B 7848	C	C	C	D	C	D	C	C	C	C	68·5	7
B 7780	C	D	D	D	C	D	C	D	D	D	46·0	2
B 7781	C	C	C	C	D	D	D	C	D	D	49·6	4
B 7786	C	D	C	C	C	C	C	D	D	D	55·8	5
B 7844	C	C	C	C	C	D	D	D	D	C	50·3	5
B 7859	C	C	C	C	D	D	C	D	D	D	48·1	4
B 7846	D	C	C	C	C	D	C	C	D	D	56·6	6
B 7787	C	C	D	D	D	D	C	D	C	C	50·7	4
B 7854	C	C	C	C	C	D	C	D	C	D	53·8	6
Ca %	60	56	59	57	60	47	57	50	49	41		

C indicates that more of the Ca diet was eaten

D indicates that more of the deficient diet was eaten

C/D indicates that equal quantities of both diets were eaten.

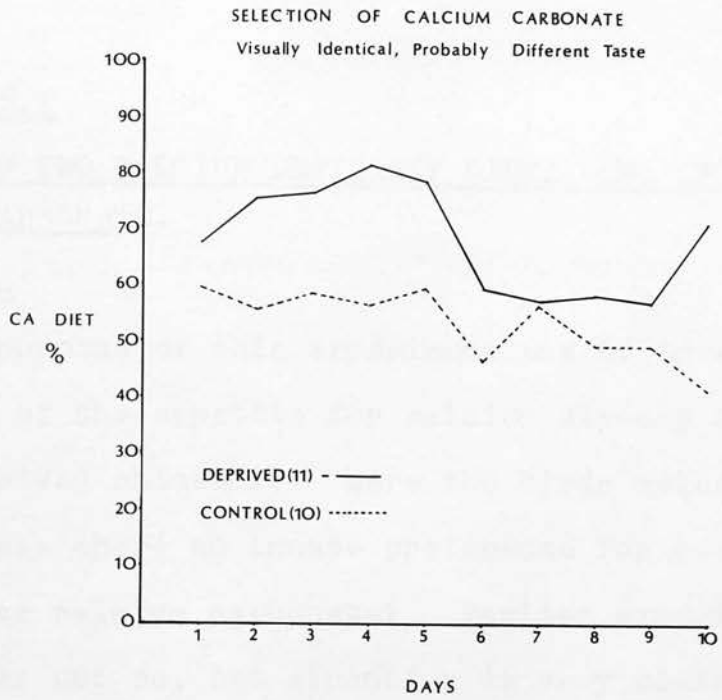


FIG. 3. Choice between a calcium-deficient diet and the same diet with 2% calcium carbonate. (Experiment 4.2)

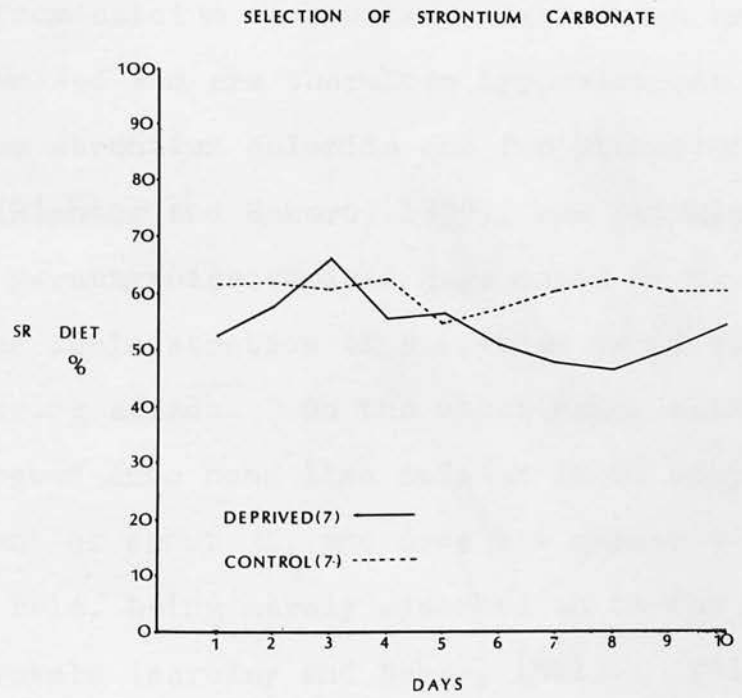


FIG. 4. Choice between a calcium-deficient diet and the same diet with 2% strontium carbonate. (Experiment 5.1)

EXPERIMENT 5.1

SELECTION OF TWO CALCIUM DEFICIENT DIETS, ONE OF WHICH CONTAINS STRONTIUM CARBONATE.

Introduction

The purpose of this experiment was to investigate the specificity of the appetite for calcium already demonstrated in calcium-deprived chickens. Were the birds selecting the calcium *per se*, or was there an innate preference for certain calcium salts such as calcium carbonate? Earlier experiments suggested that this was not so, but strontium is very closely related to calcium, and it seemed likely that any innate preference should generalise. To human taste, strontium carbonate is indistinguishable from calcium carbonate. Rats which have been parathyroidectomised and are therefore hypocalcaemic show an increased appetite for strontium chloride and for strontium lactate solutions (Richter and Eckert, 1939), and Swingle and Wenner (1926) found that parathyroidectomised dogs could be kept alive for many weeks by the administration of strontium lactate, which had a calcium-sparing effect. On the other hand, although strontium is incorporated into bone like calcium it is only exchangeable to the extent of about 3%, and does not appear to have any structural role, being merely adsorbed on to the surface of the apatite crystals (Bartley and Reber, 1961). This finding suggests that strontium should not have any beneficial effect when fed to a calcium-deficient bird.

Subjects

14 eight-week-old broiler chickens, of both sexes.

Methods

Essentially the same experimental procedure was used as in previous experiments. The birds were divided randomly into two groups of seven. The deprived group was fed a diet containing 0·1% calcium (Diet 2) and the control group a diet with approximately 3% calcium (Diet 5). After 28 days on these diets both groups were offered a choice between 2 diets. These were in the form of mash and were contained in polystyrene boxes. One diet was the basal low calcium diet and the other was the basal diet plus 2% strontium carbonate, and 200 gms. of each was presented daily. The two boxes were weighed each day, the weights were made up to 200 gms. again, and then they were switched from left to right or vice versa.

Results

These are shown in Table 8 and in full detail in 8A. Subjects were classified into four groups as before according to their performance. Those which ate more of the Sr diet on 7 or more of the last nine days were classified as preferring Sr, while those which ate more of the basal diet on 7 or more of the last nine days were classified as rejecting Sr. Those which ate consistently from the left (or the right) hand box for 8+ of the last nine days were said to show a positional preference, and those which fell into none of the above categories were placed into the "no preference" group.

The criteria correlated well when compared with the relative totals of the two diets actually consumed over the 10 day period.

	Deprived	Normal controls
Sr preference	73·6 - 85·7%	65·0 - 92·0%
Sr rejection	20·9%	42·3%
Positional Pref.	48·5 - 60·0%	51·7%
No Preference	48·6 - 54·6%	48·2 - 55·1 - 70·5%

The only disagreement occurs in the case of B 7321, which although classified as having no preference, nevertheless scores more highly (70·5%) than B 7301 (65·0%) which was said to show Sr preference. However B 7321 is a borderline case, scoring 6 out of 9, and it is impossible to avoid an arbitrary decision in this case.

The results may therefore be summarised as follows:

Group	Strontium Preference	Strontium Rejection	Positional Preference	No Preference
Experim.	2	1	1	3
Control	2	1	2	2

Using the program (called 'Conprob') written for the computer to examine goodness of fit in this form of two by four arrangement, the probability that this distribution could have occurred by chance worked out at P = 1·000.

Conclusions

It may be said therefore that small though the number of chickens in this experiment may be the results certainly suggest that calcium deprived birds show no preference whatever for strontium

carbonate. The average percentage intake of the strontium diet is shown on a daily basis in Fig. 4. It will be seen that the intakes of both deprived and control groups run quite close together over the 10 day period. The greatest separation occurs at day 8, but they have come closer together again by day 10.

Table 8

Experimental Birds (Calcium deprived)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Sr %	Sr No.
B 7317	S	D	S	S	S	S	S	S	S	S	73·6	8
B 7304	D	S	S	D	D	D	D	D	D	D	20·9	2
B 7315	D	S	S	S	S	S	S	S	S	S	85·7	9
B 7252	S	D	D	D	S	S	S	S	S	S	54·6	6
B 7308	D	S	D	S	D	S	D	S	D	S	48·4	5
B 7307	S	D	S/D	D	S	S	D	D	S	D	48·6	3½
B 7310	S	S	S	D	S	D	S	D	S	D	60·0	5
Sr %	52	58	66	56	56	51	48	47	50	54		
Controls												
B 7301	S	S	S	S	S	S	S	S	S	S	66·3	9
B 7250	S	D	S	D	S	D	S	D	S	D	51·7	4
B 7251	D	S	D	D	D	D	D	S	D	D	42·3	2
B 7248	S	S	S	D	S	D	D	D	S	S	55·1	5
B 7321	S	S	D	S	D	S	S	S	D	S	70·5	6
B 7257	S	S	S	S	S	S	S	S	S	S	92·0	9
B 7254	D	D	S	S	D	S	D	S	S	S	48·2	6
Sr %	61	61	61	63	55	57	60	63	60	60		

S indicates that more of the strontium diet was eaten

D indicates that more of the basal deficient diet was eaten

S/D indicates that equal amounts of both diets were eaten.

EXPERIMENT 5.2

Introduction

Experiment 5.1 had demonstrated that calcium-deprived chickens, unlike rats, do not show any preference for a strontium-enriched diet. However, the number of subjects was small and the strontium carbonate was being presented at a comparatively low level (2%) so it was thought desirable to repeat the experiment with a larger number of birds and to offer strontium at a higher level. In addition, it would be interesting to know exactly how much strontium was being laid down in the bones of these deprived chickens, compared with their controls.

Materials and Methods

The experiment was carried out in two parts, in basis identical to 5.1. In Part 1 15 eight-week-old broilers were divided at random into an experimental group of 8 and a control group of 7. The deprived group received the low calcium diet (Diet 2), and the controls a breeders ration with approximately 3% calcium (Diet 5). After 42 days both groups were presented with 2 boxes, one containing the low calcium mash and the other the same diet with the addition of 4% powdered strontium carbonate. Food intake of individual birds was recorded daily and the boxes switched from left to right. Part 2 was a replicate, the 18 subjects being divided into an experimental group of 10 and a control group of 8. The other difference was that the diet with 4% of strontium carbonate was coloured pink with the food dye

Ponceau 2R. Selection of these two diets was recorded for a period of 10 days.

Results

Part 1

These are given in detail in the appendix (Table 9A) and at the end of this experiment (Table 9). Summaries are shown below. Each bird was classified as showing a preference, a rejection, a positional preference or no preference, using the criteria described in Experiment 1.

Group	Sr Pref.	Sr Rej.	Pos. Pref.	No Pref.
Ca deprived	0	1	2	5
Controls	0	0	3	4

These results were evaluated statistically using the program 'Conprob', and P = 1·00, indicating that there is no significant difference between these two groups.

After the 10 day period of testing the right tibiae of all the birds were cleaned, dried and assayed for levels of strontium. They were ground up, ashed, dissolved in hydrochloric acid and strontium was estimated, using the atomic absorption spectrometer. The levels found are shown below:

Deprived Group		Control Group	
0·13%	1·10%	0·14%	0·20%
0·18%	1·38%	0·15%	0·21%
0·18%	1·53%	0·16%	1·08%
0·18%	1·56%	0·17%	

Mean = 0·78% (s.e. ± 0·15) Mean = 0·30% (s.e. ± 0·04)

(All values given are percentages of dry bone tissue)

Clearly, over the period of 10 days a certain amount of strontium has been laid down in the bones of these birds, but the level is still comparatively low. More strontium appears to have been taken up by the bones of the deprived group, but the bi-modal distribution is difficult to explain. The largest figure in the deprived group (1·56%) implies that between 5 and 6% of the bone calcium has been replaced by strontium - this figure is rather larger than that found by Bartley and Reber (1961) in pigs.

Part 2

The detailed results are to be found in Table 10A and in a shorter form in Table 10 at the end of this experiment. A summary is given below.

The birds may be classified thus:

Group	Sr Pref.	Sr Rej.	Pos. Pref.	No Pref.
Deprived	0	6	0	2
Controls	0	4	0	6

The probability that two distributions as different as this would occur by chance is: P = 0·188

When the two parts of the experiment are combined the results are as follows:

Deprived	0	7	2	7
Controls	0	4	3	10

Here P = 0·565.

Thus in neither part of the experiment is there any suggestion of a significant difference between the deprived and the

control groups. Both exhibit a marked rejection of the strontium supplemented diet, which tends to remain stable or even intensify over the 10 day period of choice.

Discussion

Again in this second part of the experiment it is quite clear that calcium deprived birds show no preference for strontium carbonate, unlike rats, which do (Richter and Eckert, 1939). Indeed, at the high level of 4% both the deprived birds and the normal controls show a rejection of the strontium supplemented diet. From the bone analysis it appears that the bones of the deprived birds have taken up more strontium than those of the normal controls, but the amount is still extremely small, being less than 1% on average. There is no real difference between Part 1, where the only difference between the diets was the presence or the absence of the strontium carbonate, and Part 2 where the red dye clearly distinguished the supplemented diet, except that in the latter the rejection was even more marked. This may have been the case because the birds did not even have to taste the diets in order to exercise their choice.

Table 9

Deprived Group

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Str %	Str No.
C 9808	S	S	D	S	D	D	D	S	D	D	40·2	3
C 9807	D	D	S	D	S	D	S	D	S	D	42·0	4
C 9798	D	S	D	S	D	S	D	S	D	S	47·6	5

[Contd.

Table 9 (Contd.)

Deprived Group (contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Str. %	Str. No.
C 9818	S	D	S	D	D	S	D	S	D	D	45·2	3
C 9813	D	D	D	D	S	D	S	S	D	S	44·4	4
C 9805	S	D	D	D	S	D	S	D	D	D	41·9	2
C 9811	S	D	S	D	S	S	S	D	S	D	52·0	5
C 9799	D	S	D	S	D	S	D	D	D	D	47·2	3
Str.%	53	42	50	44	48	54	45	45	39	36		
Control Group												
C 9800	S	D	D	S	D	S	S	S	S	D	52·4	5
C 9806	D	S	D	S	D	S	D	S	D	S	44·8	5
C 9809	D	S	D	S	D	S	D	D	D	D	33·7	3
C 9812	D	S	D	S	S	D	S	D	D	S	52·9	5
C 9802	S	S	S	D	S	D	S	D	S	D	48·3	5
C 9801	S	D	S	D	S	D	S	D	S	D	50·9	4
C 9796	S/D	D	S	D	S	D	S	D	D	D	41·3	3
Str.%	44	51	40	48	53	45	58	34	51	37		

N.B. S = More of the strontium diet was consumed
D = More of the basal deficient diet was consumed
S/D= Equal quantities of both diets were consumed.

Table 10

Deprived Group

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Str. %	Str. No.
D 100	D	D	D	D	D	D	D	D	D	D	10·6	0
D 97	D	D	D	D	D	S	D	S	D	S	37·5	3
D 91	S	D	D	D	D	D	D	D	D	D	10·9	0
D 88	D	D	S	D	D	D	D	D	D	S	39·3	2
D 89	S	D	D	D	D	D	D	D	D	D	16·9	0

[Contd.

Table 10 (Contd.)

Deprived Group (Contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Str. %	Str. No.
D 95	S	D	D	D	D	D	D	D	D	D	28·6	0
D 94	D	D	D	D	D	D	D	D	D	D	14·8	0
D 93	S	S	D	S	D	D	D	D	S	D	38·8	3
Str. %	45	32	33	27	22	19	21	24	25	25		
Control Group												
D 84	S	S	D	S	D	D	D	D	S	D	46·5	3
D 90	S	D	D	D	D	S	D	D	D	S	38·9	3
D 78	D	D	D	D	D	D	D	D	S	D	35·2	1
D 83	S	S	S	S	D	S	D	S	D	D	53·7	4
D 87	S	D	D	D	D	D	S	D	D	D	37·0	1
D 86	S	D	S	S	D	D	S	S	S	D	51·9	5
D 81	D	D	D	D	D	D	D	D	D	D	30·8	0
D 79	D	D	D	S	S	S	D	D	D	D	43·7	3
D 80	S	D	D	D	D	D	D	D	D	D	28·4	0
D 96	D	D	D	S	D	S	D	D	D	S	43·0	3
Str. %	45	43	39	45	37	49	40	39	42	35		

EXPERIMENT 6.

CALCIUM DEPRIVED BIRDS AND THEIR DISCRIMINATION BETWEEN CALCIUM CARBONATE AND STRONTIUM CARBONATE.

Introduction

Earlier experiments had shown that calcium-deprived birds showed a preference for a diet containing calcium carbonate as against a deficient diet, and also that they showed no preference for strontium carbonate versus a deficient diet, in

spite of the similarity of taste between calcium and strontium carbonate, the fact that their metabolic pathways are similar, and that they are to a very minor degree interchangeable in bone. The present experiment was designed to investigate the birds' choice when presented with two diets similar in taste, colour and texture and differing only in that one contained calcium carbonate and the other strontium carbonate.

Subjects

This experiment was done with three separate batches of chickens. All were about 8 weeks old at the beginning of deprivation. The first group consisted of 18 birds, divided at random into groups of 9 experimental and 9 control birds. The second consisted of seven birds split into groups of 3 and 4. The third consisted of fifteen birds, divided into an experimental group of seven and a control group of eight. The three batches will be combined for purposes of description however since they were all treated exactly alike.

Methods

The experimental birds were fed a diet (Diet 2) in the form of mash containing 0·03% of calcium while the controls received a breeders ration with approximately 3% of calcium (Diet 5). After 28 days on these diets both deprived and control birds were offered a choice. The procedure was as described in earlier experiments, with the two diets presented in polystyrene boxes. These diets were:

1. Calcium diet. To the basal calcium-deficient Diet 2 was added 2% of precipitated calcium carbonate, giving a level of 0·8% calcium.
2. Strontium diet. To the basal diet 2 was added 2% of strontium carbonate. As the particles of strontium carbonate were not as fine as the calcium carbonate, even after grinding in a pestle and mortar, about 1% of white flour had to be added to this diet to make it as white and as dusty as the calcium diet.

These two diets were now presented for a period of 10 days, and daily records of intake were made, the diets being switched from left to right each day.

Results

These are shown in Table 11, and in full detail in Table 11A, each batch being identified by a number. A summary of the results is given below, using the same criteria for the different classes as in earlier experiments.

Group	Ca Pref.	Ca Rej.	No Pref.	Pos. Pref.
Batch 1				
Deprived	3	1	5	-
Controls	2	-	6	1
Batch 2				
Deprived	-	-	-	3
Controls	-	1	3	-
Batch 3				
Deprived	-	1	5	1
Controls	-	-	6	2

[Contd.

Group	Ca Pref.	Ca Rej.	No Pref.	Pos. Pref.
Combined				
Deprived	3	2	10	4
Controls	2	1	15	3

These distributions were examined using the computer program 'Conprob' in order to assess the probability that they could have occurred by chance, and the probabilities were as follows:

Batch 1	Batch 2	Batch 3	Combined
P = 1·0000	P = 0·0571	P = 1·0000	P = 0·7267

There is therefore, no significant difference between the deprived group and the controls. It is true that Batch 2 approaches a significant difference, but this is not because of any calcium preference or rejection but because the deprived group showed positional preference rather than no preference. If the individual calcium intakes of the birds are classified they appear as follows:

Group	30 - 40%	40 - 50%	50 - 60%	60 - 70%
Deprived	1	5	11	2
Control	1	5	15	-

Here too there appears to be little difference, and the graph of total daily Ca diet intake confirms this impression (Fig. 5), although the deprived group shows a greater oscillation around the 50% mark than the controls.

Conclusions

When the two diets have been matched for taste, colour and texture it seems to be established that deprived chickens show

no preference for calcium. This cannot be because they are deriving no beneficial effect from the calcium, and so it must be that they are unable to relate any effect experienced to one or other of the diets. It is interesting that in Batch 2 there was almost a significant difference between the groups in terms of positional preference, because this is perhaps what one might expect when a beneficial effect is experienced but cannot be related to either diet, - but this difference was not noted in either of the other two batches. The reason for the birds failing to differentiate cannot be that strontium carbonate is equally as attractive as calcium carbonate in view of the fact that an earlier experiment showed that calcium deprived chickens show no preference for strontium carbonate.

Table 11

Deprived

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
1.												
B 7421	S	S	C	C/S	S	C	S	C	C	C	56·3	5½
B 7434	C	S	C	C	C	C	C	C	C	C	62·1	8
B 7855	S	C	C	C	S	C	C	C	C	C	56·7	8
B 7414	C	S	C	C	S	C	C	S	C	S	58·3	5
B 7426	S	S	S	C	S	S	S	S	S	S	31·0	1
B 7418	C	S	C	S	S	C	C	S	C	C	55·7	5
B 7422	C	S	C	C	C	C	C	C	C	C	68·1	8
B 7433	C	S	C	S	S	S	S	C	C	C	54·2	4
B 7431	C	S	C	S	S	S	C	S	C	S	50·1	3

[Contd.

Table 11 (Contd.)

Deprived (Contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
2.												
B 8169	S	S	S	C	S	C	S	C	S	C	46·9	4
B 8164	C	S	S	C	S	C	S	C	S	C	47·1	4
B 8168	S	S	S	C	S	C	S	C	S	C	42·1	4
3.												
B 8691	C	S	C	S	S	S	S	S	C	S	47·1	2
B 8689	C/S	C	S	C	S	C	S	C	S	C	51·2	5
P 1128	S	S	S	C	S	S	S	C	C	C	46·8	4
B 8684	C	S	S	C	C/S	C	C	C	C	S	57·5	5½
B 8167	C	S	C	C	C	C	S	S	C	S	54·4	5
B 8176	S	S	C	C	C	S	C	S	C	C	51·7	6
B 8171	C	C	S	C	C	C	S	C	S	S	51·9	5
Ca %	52	44	51	60	45	62	52	54	54	53		

Controls

1.												
B 7427	S	C	S	C	S	C	C	S	C	S	52·3	5
B 7843	S	S	S	C	C	C	S	C	S	S	46·7	4
B 7424	S	S	S	C	C	C	C	C	C	S	57·2	6
B 7420	C	S	C	S	C	S	C	C	C	S	53·6	5
B 7429	C	S	C	C	C	C	C	C	S	C	55·1	7
B 7425	C	C	C	C	C	S	C	S	S	C	58·4	6
B 7428	C	C	S	C	S	S	C	S	C	C	55·1	5
B 7412	S	C	S	C	S	C	S	C	C	C	56·2	6
B 7423	C	C	C	S	C	C	C	C	C	C	55·8	8
2.												
B 8175	C	S	S	S	S	S	S	S	S	C	37·4	1
B 8174	S	S	S	S	C	S	C	C	C	C	51·3	5
B 8161	C	S	C	S	C	C	C	S	S	C	51·6	5
B 8170	C	S	C	S	C	C	C	C	S	C	48·5	6

[Contd.

Table 11 (Contd.)

Controls (Contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
3.												
B 9098	S	C	S	C	S	S	C	S	S	C	46·5	4
B 8165	C	S	S	C	S	S	C	C	S	C	52·8	4
B 8166	S	S	S	S	S	C	C	C	S	S	48·4	3
B 8173	C	C	S	C	S	S	S	C	C	C	51·6	5
B 8930	S	C	C	C	S	C	S	C	S	C	52·3	6
B 8933	C	S	C	S	C	S	C	S	C	S	50·8	4
B 8172	S	S	S	S	C	C	C	S	S	S	41·0	3
B 8163	C	C	C	C	C	S	S	S	S	C	51·6	5
Ca %	51	52	46	53	50	52	56	51	50	52		

C = more of the Ca diet was eaten

S = more of the Sr diet was eaten

C/S = equal amounts of both diets were eaten.

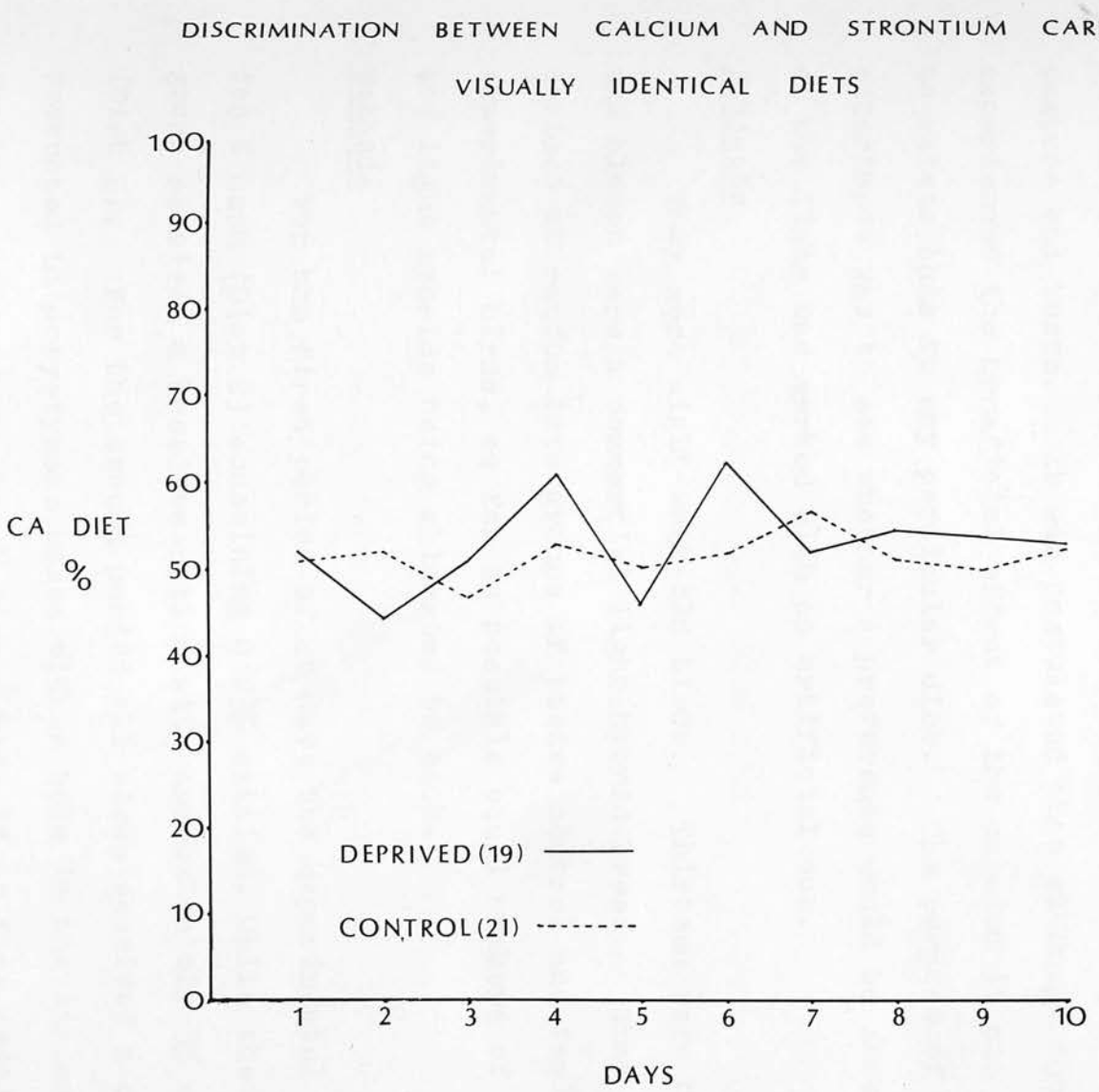


FIG. 5. Choice between diets which are similar except that one contains 2% calcium carbonate while the other contains 2% strontium carbonate. (Experiment 6)

EXPERIMENT 7.

PREFERENCE FOR CALCIUM CARBONATE WHEN DIETS ARE MATCHED FOR TASTE BUT ARE VISUALLY DIFFERENT.

Introduction

Experiment 6 had shown that calcium deprived birds showed no preference for a diet containing calcium carbonate when it was presented together with a deficient diet matched for colour, texture and taste. It was postulated that although the bird experienced the beneficial effect of the calcium it was unable to relate this to any particular diet. The purpose of this experiment was to see whether a preference would be shown if one of the diets was marked with an artificial cue.

Subjects

They were eight-week-old birds. Thirteen were broilers and eleven were a commercial light hybrid breed. They were divided at random into groups of twelve control and twelve experimental birds, as far as possible equal numbers of broilers and light hybrids being allocated to each.

Methods

For the first period of 28 days the experimental group was fed a mash (Diet 2) containing 0·03% calcium, while the control group received a breeders ration with approximately 3% calcium (Diet 5). For the second period all birds received a choice, presented in polystyrene boxes with a hole in the lid as usual. One box contained the basal Diet 2 plus 2% of fine precipitated

calcium carbonate, while the other contained Diet 2 plus 2% of strontium carbonate and sufficient Ponceau 2R solution to give a medium pink coloration (Ponceau 2R is a red food dye). Each day the boxes were weighed, made up to 200 gms. and switched from left to right. After 11 days the situation was changed - it was now the calcium diet which was dyed pink, and the strontium diet was fed in plain form. The experiment continued for a further 5 days in the case of the control group and for 7 days in the case of the deprived group. At the conclusion of the experiment plasma calcium levels were estimated.

Results

These are given in Table 12.1, and in full detail in Table 12.1A, and in summarised form below - the usual criteria being used.

Group	Ca Pref.	Ca Rej.	No Pref.	Posit. Pref.
Deprived	8	-	3	1
Controls	2	-	9	1

Using the computer program 'Conprob' the probability that this distribution could have occurred by chance was calculated to be: P = 0·0196. There is therefore a significant difference between deprived and control birds, the deprived birds showing a greater calcium preference. This result appears at first sight to be convincing enough evidence that colour difference is an adequate cue for the birds to show discrimination. When the results are examined more closely however some interesting points emerge. The graph (Fig. 6) shows that although the deprived birds in toto

had a consistently higher intake of calcium diet than the control group, the pattern of intake over the 11 days was rather different from usual. In the past the deprived group tended to have an intake of approximately 50% on the first day, which gradually rose over 5 or 6 days to approximately 80 to 90%. In this case the level started at 65% and thereafter varied between 65 and 60%, so that there appeared to be immediate preference which was maintained rather than a gradual learning process as previously. When the percentage intakes for individual birds are examined, these too tend to be somewhat lower than in previous experiments. The highest figure is 78·4%, while six fall between 60 and 70% and four between 50 and 60%. Thus though there was a preference for calcium it was not as marked or clear cut as on some previous occasions.

Conclusions

The reversal of the red dye at day 12 is followed by an almost perfect reversal of preference, - the Ca intake falling from 62% to 28%, and the number of deprived birds showing a calcium preference falling from 12/12 on Day 11 to 2/12 on Day 12. This seems to be convincing evidence that the preference shown was not for calcium as such but rather for the visual properties of the diet. However once the calcium was no longer present in the plain diet the preference for it waned and by Day 15 the total Ca percentage intake is back to 50% again. There is no suggestion on the three final days over which records were kept that a new

calcium preference is beginning to build up. Of course in any case it may be that the birds are not as calcium deficient as they had been - their average intake over the 11 days would be about 5 - 6 gms. of calcium, and the figures for plasma calcium suggest that deficiency was not now as severe (Table 12.2). One may conclude therefore that visual cues alone are adequate for calcium deprived birds to exhibit a preference, but there is some evidence that the preference is not as pronounced as it is when both visual and gustatory cues are present, and also that the preference is for some visual property of the diet rather than for calcium itself, although this preference for a particular visual cue only develops because it is linked to the presence of calcium, and once the association is broken the preference wanes.

Table 12.1

Deprived

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	11	Ca %	Ca No.
P 1132	C	C	C	C	S	S	C	C	C	S	C	53·2	6
P 1137	C	C	C	C	C	C	C	C	C	C	C	65·1	9
P 1138	C	C	S	C	C	C	C	C	C/S	C	C	67·9	7½
B 8683	C	S	C	C	C	C	C	C	C	C	C	60·6	8
B 8689	S	C	C	C	C	C	C	C	C	C	C	73·7	9
P 1141	C/S	C	C	C	C	C/S	S	C	C	C	C	63·4	7½
P 1142	C	C	C	C	C	S	C	C	S	S	C	57·3	6
B 8692	C	C	S	C	C	C	C	C	C	C	C	61·0	8
B 8665	C	C	C	S	S	S	C	C	C	C	C	56·5	6
P 1127	C	C	C	C	C	C	C	C	C	S	C	78·4	8
B 8694	C	C	C	S	C	S	C	S	C	S	C	62·3	5
B 8695	S	C	C	C	C	C	C	S	C	C	C	56·6	8
Ca %	65	68	63	60	62	60	65	60	66	59	61		

[Contd.

Table 12.1 (Contd.)

Controls

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	11	Ca %	Ca No.
B 8688	S	C	C	S	S	S	S	C	S	C	S	50·2	4
B 8687	C/S	C	S	C	S	C	S	S	C	S	C	50·3	4
P 1136	C/S	C/S	C	S	C	S	C	S	C	S	C	48·0	4½
P 1134	C/S	C	S	C	C	S	C	C	S	S	C	57·7	5
B 8666	S	C	S	C	S	S	S	C	S	C	S	51·8	4
P 1140	S	C/S	C	S	C	S	C	C	S	C	S	53·0	5½
B 8686	S	C	S	C	S	S	C	C	S	S	S	50·5	4
B 8693	S	S	C	S	C	C	C	C	C	C	S	54·6	7
P 1133	C	S	C	C	C	C	C	C	C	S	S	54·5	7
B 8667	S	C	C	C	S	S	S	C	S	S	C	44·2	4
P 1135	S	S	C	C	S	C	S	C	S	C	C	48·5	5
B 8690	S	C	C	C	C	C	C	S	S	S	S	51·4	6
Ca %	38	58	53	50	55	49	53	52	47	50	51		

After reversal of food dye

Deprived

Bird No.	Day 12	13	14	15	16	17	18	Ca %	Ca No.
P 1132	S	C	S	C	C	C	C	55·1	5
P 1137	S	S	S	S	S	S	S	3·0	0
P 1138	S	S	S	S	C	S	C	40·6	2
B 8683	S	S	S	C	S	S	C/S	40·5	1½
B 8689	C	S	C	S	C	S	C	47·2	4
P 1141	S	S	S	S	S	C	S	39·6	1
P 1142	S	S	S	S	S	C	S	38·2	1
B 8692	S	S	S	C	C	C	C	49·5	4
B 8665	S	S	S	C	C	S	C	47·1	3
P 1127	C	C	C	C	S	S	S	58·3	4
B 8694	S	C/S	S	C	S	C	S	41·5	2½
B 8695	S	S	S	C	C	S	C	46·9	3
Ca %	28	31	35	51	49	45	48		

[Contd.

Table 12. 1 (Contd.)

After reversal of food dye (Contd.)

Controls

Bird No.	Day 12	13	14	15	16	Ca %	Ca No.
B 8688	S	C	S	C	S	50·6	2
B 8687	S	S	C	S	S	40·3	1
P 1136	S	C	S	C	S	45·4	2
P 1134	C	S	C	S	C	54·0	3
B 8666	C	S	C	C	C	54·8	4
P 1140	C	C	C	C	C	75·3	5
B 8686	S	S	C	C	C	51·3	3
B 8693	S	C	S	C	S	47·0	2
P 1133	C	S	C	S	C	57·7	3
P 1135	C	S	C/S	C	S	48·9	2½
B 8690	C	S	S	S	S	48·2	1
Ca %	52	50	51	54	52		

Table 12.2

Levels of plasma calcium following choice period.

Deprived	Controls
10·42 ± 0·09 mg/100 mls	11·35 ± 0·12 mg/100 mls

(Fuller details may be found under Table 12.2A)

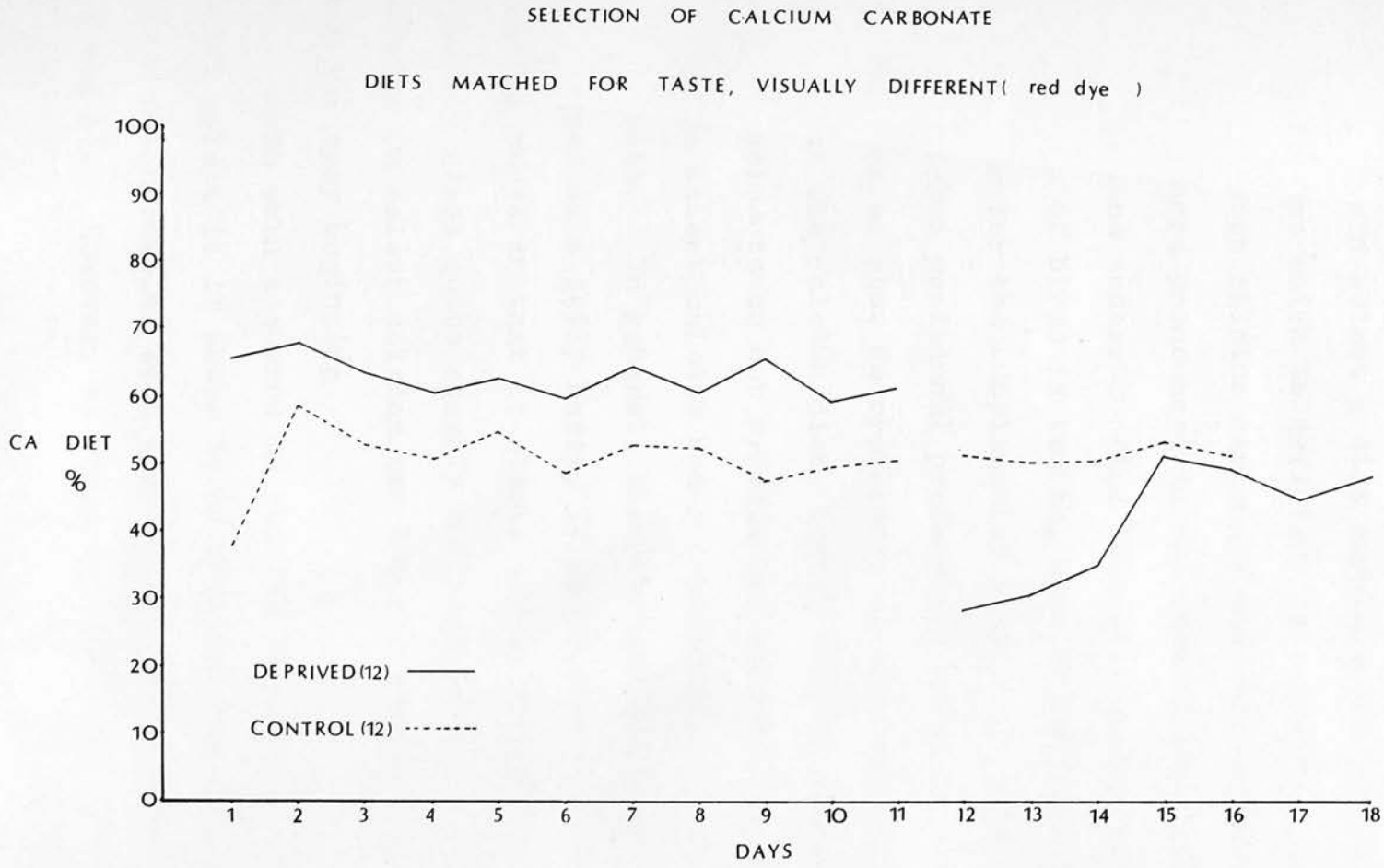


FIG. 6. Choice between two diets, one containing 2% calcium carbonate while the other contains 2% strontium carbonate. (Experiment 7)

A BRIEF SUMMARY OF THE FINDINGS CONTAINED IN EXPERIMENTS 1 TO 7

This series of experiments has confirmed that chickens, when deprived of calcium by feeding a deficient diet for a period of weeks, do indeed select a diet supplemented with calcium salts rather than one which is deficient in calcium. This is so in the case of both calcium carbonate and calcium lactate, and indeed seems to be more pronounced in the case of the latter, whereas normal chickens appear to find this salt rather aversive. Usually, when a group of birds is tested, some individuals fail to exhibit a preference for the supplemented diet. In some cases this is due to a marked positional preference, while in other cases the bird appears to show no preference at all, and on occasion it may even reject the calcium diet, though this is comparatively uncommon. These experiments do not provide any support for the idea that ability to select calcium under conditions of need is in any sense innate. In general, when the group calcium diet consumption is examined on a daily basis, it bears some resemblance to a learning curve in that it starts off at around chance levels, and initially rises quite steeply and then flattens off. If the capacity to select calcium was inborn, consumption should be high from the very beginning.

Even when a source of calcium is present, the chicken cannot select it if there is no physical cue such as flavour or colour which enables each diet to be distinguished (Experiments 4.1 and 6). However, if there is sufficient difference, the

appropriate selection can be made, whether the cue is flavour (Experiment 4.2) or colour (Experiment 7).

However, these experiments may not rule out entirely the possibility that the chicken may possess some inborn capacity to select a source of calcium when the need for it is present. In the experiments described above the calcium has always been present at a fairly low level in the diet, and it is possible that the chicken is unable to recognise it as such. Lorenz (1966) has written "We know of one case at least in which a special releasing mechanism achieves the recognition of a needed chemical: birds lacking calcium will peck at and eat any white, hard, and crumbling substance, regardless of its chemical composition, obviously guided by visual and tactile stimuli rather than by chemical ones." Before innate factors can be ruled out entirely therefore it is important that the chicken should be tested in an environment where it can be presented with a wider range of stimuli, and compared with controls to see whether calcium deprivation alters its responses in the way that Lorenz suggests.

EXPERIMENT 8.1

THE BEHAVIOUR OF CALCIUM DEFICIENT CHICKENS WHEN EXPOSED TO VARIOUS STIMULI IN AN OPEN FIELD SITUATION.

Introduction

Calcium deficient birds were compared with normal controls in their pecking responses to small objects, to see whether there was a difference in pecking rate and pattern. The experiment was performed on three separate batches of birds, in three parts.

Part 1

Subjects

Twelve broiler chickens of both sexes - about 11 weeks old at time of testing.

Methods

The birds were divided at random into 2 groups of six birds each at the age of 7 weeks, and placed in adjacent cages in the battery house. The controls were given a commercial ration containing 3% Ca (Diet 5) while the experimental birds were given Diet 2 with 0·1% calcium. Both these rations were in the form of pellets. No cockle shell or grit of any kind was offered to either group of birds. At the end of 26 days, during which food and water were available ad lib., all the birds were tested. The testing area was an arena, six feet square, with a grid of reference lines one foot apart in white paint on the floor, and with 18 inch high plywood walls. Across one corner was placed a row of four petri dishes, containing flint grit, cockle shell, perspex

chips and coal chips, respectively. All the fragments were approximately the same size - about 1/8 inch across and the dishes were brightly lit from above. These four particular substances were selected because they covered a broad spectrum. Cockle shell is white and dull, flint is grey and moderately shiny, coal is black and shiny and perspex is transparent and shiny. The birds were placed in the arena, one at a time, about one foot away from the dishes and facing them - see diagram. They were left there for three minutes exactly and then returned to their cage. Any droppings were removed as far as possible before the next bird was tested, but owing to the roughness of the concrete floor complete removal was not always achieved. All the controls were tested first, then all the experimental birds, then all the controls again in the same order, and finally all the experimental birds again. After each bird had been tested any chips which had been split were replaced.

Results

These are shown in Table 13, both first and second trials being shown for each bird. Birds were scored primarily for pecks, though in this experiment any birds which showed interest in a dish without pecking it were also scored. However in the later experiments this was not noted, owing to the subjective nature of this measure which appeared to add little information.

It will be seen that only one control bird on one occasion showed even a fleeting interest in any of the dishes, and this interest was not repeated on the second trial. Otherwise the

controls were occupied in walking around the arena, apparently searching for an avenue of escape. They showed no interest in the grit, although they had previously had no access to any.

The experimental birds, apart from B 5375, all showed interest on the first trial in at least one of the dishes, three of them pecking, and on the second trial all (again excepting B 5375) pecked at one or more of the dishes. The largest number of pecks counted was 207 - which is a rate of more than one a second and involved almost continuous pecking throughout the period. In addition to the dishes and their contents these experimental birds pecked at a variety of other stimuli - notably at white spots and irregularities on the floor, and at residual fragments of the droppings of earlier birds (and including their own) - but these pecks were not scored. The reason for not scoring this pecking was partly because the stimulus situation varied from bird to bird and partly because much of the floor pecking occurred when the bird was facing away from the experimenter, and so could not be accurately scored.

The only bird whose behaviour was atypical was B 5375 - it remained motionless on the spot where it had been placed throughout the two 3 minute periods. After being disturbed at the end of the period it pecked with frantic urgency at whichever of the dishes was closest. It seemed probable that it was in the trance-like state which is produced by fear, but nevertheless it had to be given a score of nil within the terms of the experiment.

The results were assessed for significance using the Mann-Whitney U test, (Siegel, 1956). In this test the subjects are placed in rank order according to the magnitude of their scores.

Bird No.	Class	Score	U	
B 5369	C	-		
B 5371	C	-		
B 5364	C	-		
B 5375	E	-		
B 5365	C	-	1	
B 5374	C	-	1	
B 5373	C	±	1	
B 5372	E	47		
B 5273	E	52		
B 5363	E	79		Thus U = 3
B 5370	E	96		
B 5367	E	144		
B 5366	E	287		

From the appropriate tables one may calculate that when U = 3 the probability of the above arrangement occurring by chance is P = ·008.

Conclusion

One may conclude that the tendency of calcium deficient chickens to peck at novel stimuli is significantly greater than that of controls. As to the relative attraction of the various stimuli, it appears that flint grit is the most popular by a clear margin, while cockle shell and coal were roughly on a par and perspex was the least attractive. The number of birds however is too small to allow a statistical conclusion to be reached on this point, and so in order to accumulate further information additional experiments were carried out.

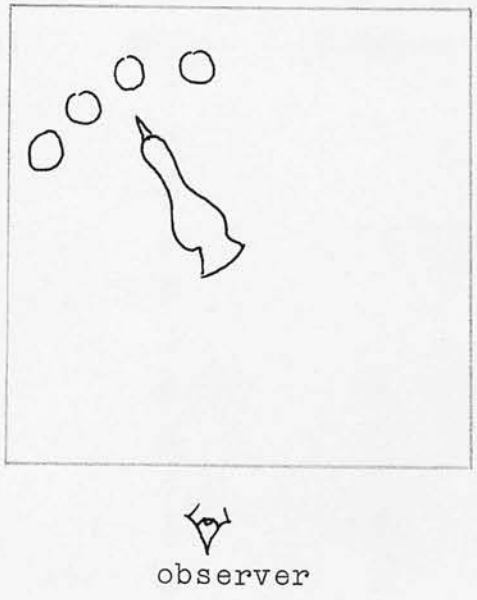
Table 13

No. of bird	Flint	Cockle Shell	Coal Chips	Perspex Chips	Total
Deprived					
B 5273	+	-	-	-	
	50	-	2	-	52
B 5363	+	-	-	-	
	63	-	12	4	79
B 5372	-	-	18	+	
	29	-	-	-	47
B 5375	-	-	-	-	
	-	-	-	-	0
B 5366	80	-	-	-	
	207	-	-	-	287
B 5367	12	+	+	++	
	129	3	-	-	144
B 5370	-	+	-	-	
	-	96	-	-	96
Controls					
B 5369	-	-	-	-	
	-	-	-	-	0
B 5371	-	-	-	-	
	-	-	-	-	0
B 5364	-	-	-	-	
	-	-	-	-	0
B 5373	-	-	-	±	
	-	-	-	-	0
B 5365	-	-	-	-	
	-	-	-	-	0
B 5374	-	-	-	-	
	-	-	-	-	0

The figures refer to the number of pecks.

Code: - No interest
± Fleeting inspection
\+ Attentive fixation
++ Very close inspection without pecking

Diagram of the typical arrangement of the experimental arena.



EXPERIMENT 8.2.

Materials and Methods

This second part of the experiment was carried out in the same way as the first. 22 eight-week-old broilers were divided into two groups of eleven, and the experimental group was fed the low calcium diet, while the control group received the normal ration containing 3% calcium. After 28 days of deprivation, exactly the same testing procedure was used as before, except that pecks to the floor were scored in addition.

Results

These are given in Table 14, and essentially confirm the findings of experiment 8.1. Only one of the deprived birds failed to peck at all, while only two out of the ten normal controls pecked, and in both cases it was at the floor. The

results can be ranked for the Mann-Whitney U test as follows:

Bird No.	Score (pecks)	Group	U value
B 7789	0	C	
B 7848	0	C	
B 7780	0	C	
B 7781	0	C	
B 7852	0	E	
B 7786	0	C	1
B 7844	0	C	1
B 7846	0	C	1
B 7787	0	C	1
B 7859	3	C	1
B 7854	8	C	1
B 7788	11	E	
B 7790	43	E	
B 7858	49	E	
B 7430	52	E	
B 7784	81	E	
B 7789	93	E	
B 7853	147	E	
B 7849	181	E	
B 7783	226	E	
B 7857	275	E	

Thus U = 6. This is significant at the ·001 level. The distribution of pecks by the birds is summarised below:

	Flint	Perspex	Coal	Shell	Floor
Total No. of pecks	434	18	185	455	67
Number of bouts	9	3	5	9	7
No. of pecks/bout	48·2	6·0	37·0	50·5	9·6

The comparable figures for the birds in Experiment 8.1 are:

Total No. of pecks	570	4	32	99
Number of bouts	7	1	3	2
No. of pecks/bout	81·4	4	10·7	49·5

Conclusions

This experiment yields results very similar to those obtained in Experiment 8.1. The deprived group peck more than the normal group and the difference is highly significant ($P < \cdot 001$). As for the relative preference shown for the different stimuli, here again the results are similar to those of Experiment 8.1 except that now cockle shell ranks more or less equal first with flint grit, instead of coming second as before. Coal and perspex were clearly less attractive, and ranked third and fourth respectively in both cases. In general, there were more pecks per bout in the case of flint and cockle shell - this implies that once pecking had begun it was more likely to continue.

Table 14

Bird No.	Flint	Perspex	Coal	Shell	Floor	Total
Deprived						
B 7430	8	-	1	1	11	
	2	13	-	1	15	52
B 7790	-	-	-	-	-	
	-	-	-	35	8	43
B 7849	-	-	-	14	21	
	-	-	6	140	-	181
B 7852	-	-	-	-	-	
	-	-	-	-	-	0
B 7789	-	-	-	-	2	
	-	-	87	-	4	93
B 7857	-	-	-	82	-	
	143	4	-	46	-	275
B 7853	4	-	-	-	-	
	143	-	-	-	-	147
B 7858	-	-	-	-	-	
	49	-	-	-	-	49

[Contd.

Table 14 (Contd.)

Bird No.	Flint	Perspex	Coal	Shell	Floor	Total
Deprived (Contd.)						
B 7788	-	-	-	-	-	
	4	1	-	6	-	11
B 7784	-	-	-	-	-	
	-	-	81	-	-	81
B 7783	14	-	-	26	6	
	67	-	9	104	-	226
Controls						
B 7859	-	-	-	-	3	
	-	-	-	-	-	3
B 7854	-	-	-	-	4	
	-	-	-	-	4	8

The remaining birds, namely: B 7779, B 7848, B 7780, B 7781, B 7786, B 7844, B 7846, B 7787, did not peck at any of the stimuli nor at the floor.

EXPERIMENT 8.3.

Material and Methods

In this, the third experiment in the series, the procedure was altered. Instead of using the large 6' square arena, a small arena of about 3'6" square was used, and in place of the two 3 minute long trials previously given to each bird it only received one trial, but this was preceded by a ten minute period of acclimatisation in the empty arena. As before the birds were scored for pecks at the stimulus dishes and also at the floor. Twenty two birds were divided into two groups, and the experimental

group was deprived of calcium by feeding a deficient diet for a period of 28 days. The deprived and control birds were acclimatised and tested alternately.

Results

These are given in Table 15, and are summarised below:

Deprived	Flint	Perspex	Coal	Shell	Floor	Total
No. of pecks	288	0	24	188	24	524
Number of bouts	6	0	3	5	3	17
No. of pecks/bout	48·0	0	6·0	37·4	6·0	
Normal						
No. of pecks	39	2	4	22	16	83
Number of bouts	6	1	1	3	2	13
No. of pecks/bout	6·5	2	4	7·3	8	

The results for each bird were then scored according to the total number of pecks and ranked in ascending order of magnitude, in order to examine them for significance using the Mann-Whitney test.

0 0 0 0 0 0 0 4 4 5 6 9 13 15 17 27 32 59 88 91 98 140

C C E E E C C C C E C C C C E E C E E E E E

U was calculated - counting the number of experimental birds that preceded each control. U = 34. When the tables are consulted ($n_1 = n_2 = 11$) this value of U is in fact the critical value for significance at the 5% level (one tailed).

Discussion

Whereas in experiments 8.1 and 8.2 none of the control birds had pecked at any of the stimuli, in the present experiment an almost equal number of both groups pecked, - in fact 7 out of 11

controls and 8 of 11 deprived birds. Nevertheless, if it is legitimate to interpret the statistical test on a one tailed basis, there was still a significant difference between the groups in that the deprived birds pecked more. There seem to be good reasons to use the one-tailed test, for one would certainly predict from the two earlier experiments that the deprived birds should peck more than the controls. In point of fact the reason why there was less difference between the two groups in this experiment is not that the deprived birds pecked less but that the controls pecked more. The most probable reason for the altered behaviour of the controls (compared with the earlier experiments) lies in the change of size of the arena. In the larger arena most of the control birds showed some form of escape behaviour - usually walking around the periphery and occasionally attempting to leap over the side. In the small arena however they were unable to get very far from the dishes and when they walked past they pecked at the chips provided. There was a subjective difference in the way the normal and the deprived birds pecked. The controls pecked in a rather casual way without showing much interest, while the deprived birds pecked with much more intensity, more rapidly in most cases and more vigorously. This is borne out by the fact that the total number of pecking bouts is fairly similar in the two groups (deprived 17, controls 13) but that the number of pecks per bout is generally less in the case of the controls, indicating that they lost interest sooner.

However, the fact that the control birds did peck to an appreciable extent in this experiment is very useful in that it gives us an indication of the preferences of the normal bird. The preference order was:

	Flint	Shell	Floor	Coal	Perspex
Normal	1	2	3	4	5
Deprived	1	2	=3½	=3½	5

The Kendall rank correlation coefficient (Siegel, 1956) was used to determine whether two distributions as similar as this were likely to have arisen by chance. When the test is applied S = 9 and P = ·025, implying that this similarity is unlikely to be a chance one.

Conclusions

Once again, as in experiments 8.1 and 8.2 the deprived birds peck more than the controls, although in this case the difference is barely significant, possibly for the reasons described. In all three experiments the order of preference is much the same - flint and shell are both highly preferred, with flint perhaps slightly more attractive, then comes pecking at the floor or at the coal chips, and finally the perspex chips are apparently an unattractive stimulus. One may conclude therefore that the effect of the calcium deprivation is to increase the level of pecking, but that it has little or no effect on the pattern of preference. Lorenz's observation is not borne out in this case, and one can certainly not conclude from these findings that there is a releaser mechanism in chickens which potentiates pecking at hard, white objects.

Table 15

Bird No.	Flint	Shell	Coal	Perspex	Floor	Total
Deprived						
B 7421	94	46	-	-	-	140
B 7785	23	-	-	-	4	27
B 7434	-	-	-	-	5	5
B 7419	-	17	-	-	-	17
B 7855	18	56	14	-	-	88
B 7414	-	-	-	-	-	0
B 7426	-	-	-	-	-	0
B 7418	6	30	8	-	15	59
B 7422	56	40	2	-	-	98
B 7433	91	-	-	-	-	91
B 7431	-	-	-	-	-	0
Controls						
B 7427	-	-	-	-	-	0
B 7843	9	-	-	-	-	9
B 7856	3	1	-	-	-	4
B 7850	3	-	-	-	12	15
B 7420	18	12	-	2	-	32
B 7424	4	-	-	-	-	4
B 7429	-	9	4	-	-	13
B 7425	2	-	-	-	4	6
B 7428	-	-	-	-	-	0
B 7412	-	-	-	-	-	0
B 7423	-	-	-	-	-	0

EXPERIMENT 8.4

THE EFFECT OF PARATHORMONE UPON 'EXPLORATORY' BEHAVIOUR

Introduction

In Experiment 8.1 it was shown that there were observable differences in behaviour between calcium deprived chickens and normal controls. The deprived birds show more pecking activity in an open arena. The deprived birds had lower plasma calcium levels than the controls but there were other differences too which might have had some influence upon the behavioural changes. In the calcium deprived birds the parathyroid glands are enlarged and turgid, and there is a high level of circulating parathyroid hormone which mobilises calcium from the bones, leading eventually to osteoporosis. The present experiment was designed to investigate the possibility that an alteration in the level of circulating parathyroid hormone itself might have a direct or an indirect

effect upon this 'exploratory' behaviour in chickens. Other hormones are known to have a profound influence on a variety of behaviour patterns (Beach, 1965; Rothchild, 1965).

Subjects

Twenty four 7-8 week old chickens were used. Twenty were of a light hybrid type and four were broilers. They were randomly divided into two groups, each consisting of ten light hybrids and two broilers.

Materials and Methods

On Day 0 the deprived group was placed on a diet adequate in every respect except that it contained only 0·03% calcium (Diet 2). The control group was fed a layers ration with approximately 3% calcium (Diet 5). On Day 13 blood samples were taken from 6 of the deprived and 6 of the control group, and the plasma calcium levels were estimated. On Day 14 both deprived and control groups were divided into subgroups, and each was assigned to a parathormone ('P') or a no treatment ('N.T.') group. Each subgroup consisted of 5 light hybrids and 1 broiler. Every bird in the 'P' group was given an injection of 1 ml/kg of Parathormone subcutaneously at 9.00. The N.T. group were similarly given a saline injection.

At 14.00 the birds were placed singly in the open field (6') for a period of 3 mins. with access to dishes filled with oyster shell, flint, coal and perspex chips and they were scored for any pecks made at the dishes or their contents, or at the floor. The groups

were tested in the following order: (D = deprived, C = Normal Control) D P, C P, D P, C P, D NT, C NT, D NT, C NT. Thus every bird was tested twice, and they were tested in this order so as to avoid an excessive delay between the 2 testings of the birds in the P groups. After testing had been completed they were returned to their cages and fed with the same diets as before.

On Day 28 the same procedure was carried out as that on Day 14 except that parathormone injections were given at 9.00 and testing was begun at 11.00 as studies suggested that parathormone exerted its greatest effect upon plasma calcium some 2 - 3 hours following subcutaneous injection (Hertelendy, 1962). Testing was complete by 15.00 and 5 birds of the deprived NT group were bled (for plasma calciums). After bleeding, two of these were given 1 ml/kg Parathormone subcutaneously and a further blood sample was taken at 17.00.

Results

They are given in Tables 17 and 18 at the end of this experiment.

These results were examined for significance using the Mann-Whitney U test, comparing the birds on the basis of the number of times they pecked.

Day 14. Deprived v. Control

Score 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 3 4 21 229

Group D D D D D D D C C C C C C C C C C C C D D D D D

Here U = 45·5 (For P = ·05 U must equal 42 or less). The deprived group pecked more than the controls.

Day 28. Deprived v. Control

Score 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 4 6 21 29 136 312

Group D D D D D D D C C C C C C C C C C D C D C D D D

Here U = 52 (Again for P = ·05 U must equal 42 or less). The deprived group pecked more than the controls.

Day 14. Deprived. Parathormone v. No Treatment

Score 0 0 0 0 0 0 0 1 3 4 21 229

Group P P NT NT NT NT NT P P P P NT

Here U = 11 and P = ·31 (two-tailed)

The Parathormone group pecked more than those in the No treatment group.

Day 14. Normal. Parathormone v. No Treatment

Score 0 0 0 0 0 0 0 0 0 0 0 1

Group P P P P P P NT NT NT NT NT NT

There is obviously no difference between these two groups.

Day 28. Deprived. Parathormone v. No Treatment

Score 0 0 0 0 0 0 0 2 6 29 136 312

Group P P P P NT NT NT P P NT NT NT

Here U = 12 and P = ·39 (two-tailed)

The No Treatment group pecked more than the Parathormone group.

Day 28. Normal. Parathormone v. No Treatment

Score 0 0 0 0 0 0 0 0 0 0 4 21

Group P P P P P NT NT NT NT NT P NT

Clearly there is no difference between these two groups.

Plasma calcium levels

Day 14	Deprived		Normal	
	No. of bird	mg/100 ml.	No. of bird	mg/100 ml.
	P 1251	10·4	P 1263	10·8
	P 1252	10·2	P 1262	12·8
	P 1255	10·0	P 1258	11·0
	P 1272	10·2	P 1256	11·6
	P 1259	10·2	P 1273	11·2
	P 1261	10·2	P 1266	11·6
	Mean = 10·2 (s.e. ± 0·057)		Mean = 11·5 (s.e. ± 0·264)	

Day 28	Deprived		
	No. of bird	mg/100 ml.	2 hrs. after 1 ml. Parathormone
	P 1268	9·2	9·8
	C 747	9·6	9·4
	P 1292	10·2	
	C 746	8·5	
	P 1281	10·2	
	Mean = 9·54 (s.e. ± 0·29)		

Discussion

There is no significant difference between deprived and control birds in the amount of pecking directed towards the stimuli in tests at either Day 14 or at Day 28 - although it approaches significance at Day 14. This is in contrast to the findings of Experiment 8.1, but the reason appears fairly clear - the extreme emotionality of the light hybrids comprising the bulk of the experimental subjects. Most "froze" as soon as they were placed

in the arena and remained immobile until the 3 min. period was over and the experimenter approached, when they showed panic-stricken escape behaviour. Amongst the deprived group, the two broilers gave 235 pecks on the first trial and 448 on the second. The corresponding figures for the ten light hybrids were 26 and 37. This failure to demonstrate in the first place a clear difference between the deprived and the normal birds is unfortunate because it throws some doubt on the general validity of the findings as regards the effect of Parathormone.

If high levels of Parathormone were a factor in the increase in pecking seen in deprived birds - it might be expected that the injection of the hormone into normal controls would raise the amount of pecking seen in these birds. On the other hand if it was the level of circulating calcium which was important the injection of Parathormone by raising blood calcium should lead to a reduction of pecking in deprived birds. It is also possible that there might be an interaction between the effect of the hormone and the blood calcium levels, and for this reason the experiment was carried out twice - at 14 and at 28 days of deprivation.

In the event Parathormone had no significant effect upon pecking in either the deprived or the normal groups, at 14 or at 28 days. This at first sight fails to support either hypothesis mentioned above - but on closer examination it is doubtful whether Parathormone does in fact raise blood calcium levels at

all in deprived birds. After 1 ml. of Parathormone the plasma calcium level rose in one bird from 9·2 mg % to 9·8 mg % and fell in another from 9·6 mg % to 9·4 mg %. Possibly in these deprived birds calcium is already being mobilised at the maximum rate and a further increase in hormone has no appreciable effect. Purely as a means of obtaining a little additional information the second of these birds (C 747) was placed in the arena a third time 2 hours after it had received 1 ml. of Parathormone and its pecking pattern was as follows:

Flint	Perspex	Shell	Coal	Floor	Total
45	-	-	45	-	90

Although there is a slight reduction in number of pecks (on previous trials 116, 196) this is broadly in line with the earlier trials and in any event the plasma calcium appeared to have if anything fallen in this bird. It appears therefore that this experiment cannot yield useful information about the second hypothesis as Parathormone does not raise the level of plasma calcium in deprived birds. This statement however is based upon the findings in two birds, and clearly it would be desirable to repeat the experiment with a batch of less emotional birds. This was indeed done, and the results may be seen under Experiment 8.5.

Table 17

Open Field Testing Day 14

No. of bird	PTh	Flint	Perspex	Shell	Coal	Floor	Total pecks
Deprived							
P 1251	-	- -	- -	- -	- -	- -	0
P 1252	+	- -	- -	- -	- -	4 -	4
P 1255	+	- -	- -	- -	- -	- -	0
P 1272	+	- -	- -	- -	- -	6 15	21
P 1259	+	- -	- -	- -	- -	- -	0
P 1261	+	- -	- -	- -	- -	1 -	1
P 1260	-	- -	- -	- -	- -	- -	0
C 503	+	- -	- -	- -	- -	1 2	3
P 1268	-	- -	- -	- -	- -	- -	0
C 747	-	- 199	- -	- -	- -	30 -	229
P 1279	-	- -	- -	- -	- -	- -	0
P 1292	-	- -	- -	- -	- -	- -	0

[Contd.

Table 17 (Contd.)

No. of bird	PTh	Flint	Perspex	Shell	Coal	Floor	Total pecks
Controls							
P 1263	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1262	-	-	-	-	-	-	0
		-	-	-	-	-	
P 1258	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1256	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1273	-	-	-	-	-	-	0
		-	-	-	-	-	
P 1266	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1253	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1263	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1270	-	-	-	-	-	-	0
		-	-	-	-	-	
P 1274	-	-	-	-	-	-	0
		-	-	-	-	-	
C 504	-	-	-	-	-	-	0
		-	-	-	-	-	
C 500	-	-	-	-	-	-	1
		-	-	-	-	1	

Table 18

Open Field Testing Day 28

No. of bird Deprived	PTh	Flint	Perspex	Shell	Coal	Floor	Total Pecks
P 1251	-	-	-	-	-	-	0
		-	-	-	-	-	
P 1252	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1255	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1272	+	-	-	-	-	2	2
		-	-	-	-	-	
P 1261	+	-	-	-	-	5	6
		-	-	-	-	1	
P 1260	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1259	+	-	-	-	-	-	0
		-	-	-	-	-	
C 747	-	116	-	-	-	-	312
		32	-	160	-	4	
P 1292	-	-	-	-	-	-	0
		-	-	-	-	-	
C 746	-	-	-	-	-	56	136
		25	-	-	24	31	
P 1281	-	-	-	-	-	2	29
		4	-	-	-	23	
P 1268	-	-	-	-	-	-	0
		-	-	-	-	-	
Controls							
P 1263	+	-	-	-	-	4	4
		-	-	-	-	-	
P 1262	-	-	-	-	-	-	0
		-	-	-	-	-	

[Contd.

Table 18 (contd.)

No. of bird	PTh	Flint	Perspex	Shell	Coal	Floor	Total Pecks
Controls (Contd.)							
P 1258	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1256	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1273	-	-	-	-	-	13	21
		-	-	-	-	8	
P 1266	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1253	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1267	+	-	-	-	-	-	0
		-	-	-	-	-	
P 1270	-	-	-	-	-	-	0
		-	-	-	-	-	
C 500	-	-	-	-	-	-	0
		-	-	-	-	-	
P 1274	-	-	-	-	-	-	0
		-	-	-	-	-	
C 504	-	-	-	-	-	-	0
		-	-	-	-	-	

EXPERIMENT 8.5.

THE EFFECT OF PARATHORMONE UPON EXPLORATORY BEHAVIOUR

Introduction

This experiment is essentially a replicate of Experiment 8.4 with a number of minor differences.

Subjects

Twenty four 8-9 week old broiler chickens were divided at random into a Deprived group of twelve birds and Control group of twelve.

Materials and Methods

On Day 0 the deprived group was placed on Diet 2, which was adequate in every respect except that it contained only 0·08% calcium. The controls were fed Diet 5 with approximately 3% calcium. On Day 32 both deprived and control groups were divided into sub-groups and each was assigned to a Parathormone ('P') or a no treatment ('N.T.') group. Blood samples were taken from representative birds.

Every bird in the 'P' groups was given an injection of 1 ml. parathormone subcutaneously at 12.00, while the N.T. groups were similarly given a saline injection. At 14.00 the birds were placed as before in the open field for 3 min. periods and scored for any pecks made at the dishes or their contents, or at the floor. They were tested in the same order as in Experiment 8.4.

On Day 34 this procedure was repeated with the deprived birds only - except that the birds which had received saline before now received

1 ml. parathormone, while those which had received parathormone now were given saline. Testing was carried out as before, the groups being tested in the order D.P. D.N.T. D.P. D.N.T. On Day 35 six of the deprived birds which had consumed little or no shell (a calcium source) were bled and were then each given 1 ml. parathormone subcutaneously at 10.00. A further blood sample was then taken from each bird two hours later, at 12.00.

Results

These are shown in Tables 19 and 20 at the end of the experiment. These results were examined for significance using the Mann-Whitney U test.

Day 32. Deprived v. Control

Score 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 5 6 23 52 133 184 159 320

Group C C C C C C C C C D D D C C D D C D D D D D D D

Here U = 26·5

So P is less than ·02. The deprived birds pecked more.

Day 32. Controls. Parathormone v. No Treatment

Score 0 0 0 0 0 0 0 0 0 1 1 5

Group P P P P P NT NT NT NT P NT NT

Here U = 14·5 (P = ·64).

Day 32. Deprived. Parathormone v. No Treatment

Score 0 0 0 1 1 6 23 52 133 159 184 320

Group P P NT P P P NT NT NT NT P NT

Here U = 9.

So P = ·18. The "no treatment" birds pecked more.

Day 34. Deprived. Parathormone v. No Treatment

Score	0	1	2	7	15	16	68	129	215	232	237	264
Group	P	NT	P	NT	NT	NT	NT	P	P	NT	P	P

Here U = 13

So P = ·48. The Parathormone-treated birds pecked more.

These results may be summarised as follows:
There was a clear and significant difference between the deprived birds and the controls in the amount of pecking observed during the 3 min. period. This confirms the result of Experiment 8.1 and suggests that the reason why this result was not obtained in Experiment 8.4 was indeed because light hybrids were used rather than, as in this experiment, broilers. On Day 32 no significant difference was seen among the controls between the birds which received parathormone and those which received saline. In the deprived group, those which had received parathormone pecked slightly less than the saline group (on Day 32) but the difference did not approach significance, while on Day 34 the Parathormone group pecked more, the difference again not being significant. Thus, one may conclude that parathormone has no effect on the particular behaviour being observed - either in normal or in calcium deprived birds. An examination of the plasma calcium levels both before and after the administration of 1 ml. of parathormone shows that the expected elevation of plasma calcium levels does not occur in calcium deprived birds (Table 21). This may be because the bird has already very high levels of endogenous

parathormone, calcium is being mobilised from the bones to the greatest possible extent, given the fact that by this time reserves are becoming short, and so the injection of additional exogenous parathormone can have little effect. It may be relevant that parathormone is ineffective in reducing the appetite for calcium seen in deficient rats unless exceedingly large doses are given (Richter and Birmingham, 1941). Apart from the fact that such large doses (5 mls. parathormone for a chicken) are toxic, they could not be used in the fowl because of the prohibitive cost.

Conclusions

One may conclude therefore that the altered behaviour seen in calcium deprived birds is not produced by the effect of elevated parathyroid hormone levels acting directly. The second possibility, that it is the central effect of lowered plasma calcium levels, must remain not proven, in view of the failure to raise these levels by the administration of parathormone.

Table 19

Open Field Testing. Day 32

Deprived Group

No. of bird	PTh	Flint	Perspex	Shell	Coal	Floor	Total pecks
C 1941	P	1	-	-	-	-	1
		-	-	-	-	-	
C 1963	P	-	-	-	-	-	0
		-	-	-	-	-	

[Contd.

Table 19 (Contd.)

Deprived Group (Contd.) No. of bird	PTh	Flint	Perspex	Shell	Coal	Floor	Total pecks
C 1943	P	- -	- -	- -	- -	1 -	1
C 1945	P	- -	- -	- -	- -	- 6	6
C 1940	P	46 68	- 11	- -	- -	34 25	184
C 1958	P	- -	- -	- -	- -	- -	0
C 1822	-	2 19	- -	- -	2 7	51 78	159
C 1944	-	- 58	- -	- -	- -	3 72	133
C 1819	-	1 2	- -	- -	- -	16 4	23
C 1816	-	- 2	- -	- -	1 -	11 39	52
C 1824	-	- -	- -	- -	- -	- -	0
C 1949	-	61 87	13 74	85 -	- -	- -	320
Control Group							
C 1962	-	- -	- -	- -	- -	- 1	1
C 1953	-	- -	- -	- -	- -	- -	0
C 1827	-	- -	- -	- -	- -	- -	0
C 1817	-	- -	- -	- -	- -	- -	0

[Contd.

Table 19 (Contd.)

Control Group (Contd.)

No. of bird	PTh	Flint	Perspex	Shell	Coal	Floor	Total pecks
C 1965	-	-	-	-	-	3	5
		-	-	-	-	2	
C 1823	-	-	-	-	-	-	0
		-	-	-	-	-	
C 1954	P	-	-	-	-	-	0
		-	-	-	-	-	
C 1959	P	-	-	-	-	-	1
		-	-	-	-	1	
C 1820	P	-	-	-	-	-	0
		-	-	-	-	-	
C 1818	P	-	-	-	-	-	0
		-	-	-	-	-	
C 1950	P	-	-	-	-	-	0
		-	-	-	-	-	
C 1821	P	-	-	-	-	-	0
		-	-	-	-	-	

Table 20

Open Field Testing. Day 34.

Deprived Group

No. of bird	PTh	Flint	Perspex	Shell	Coal	Floor	Total pecks
C 1941	-	-	-	-	-	1	1
		-	-	-	-	-	
C 1963	-	-	-	-	-	-	15
		14	-	-	-	1	
C 1943	-	-	-	-	-	-	7
		7	-	-	-	-	
C 1945	-	-	-	-	-	15	16
		-	-	-	-	1	

[Contd.

Table 20 (Contd.)

Deprived Group (contd.)

No. of bird	PTh	Flint	Perspex	Shell	Coal	Floor	Total pecks
C 1940	-	54	3	14	4	36	232
		17	-	12	-	92	
C 1958	-	34	-	-	-	7	68
		12	-	-	7	8	
C 1822	P	90	-	16	4	2	215
		52	-	-	34	17	
C 1944	P	94	-	4	2	22	264
		88	-	-	30	24	
C 1819	P	-	-	-	-	2	2
		-	-	-	-	-	
C 1816	P	-	-	-	28	9	129
		-	-	-	85	7	
C 1824	P	-	-	-	-	-	0
		-	-	-	-	-	
C 1949	P	55	-	70	10	-	237
		-	-	75	-	27	

Table 21

Plasma Calcium Levels

Blood samples taken on Day 35

No. of bird	Initial Ca level mg/100 ml.	Level 2 hrs after 1 ml. PTh mg/100 ml.
C 1941	9·6	9·4
C 1943	10·1	10·2
C 1945	9·8	9·4
C 1958	9·4	9·8
C 1816	9·6	8·0
C 1824	7·2	8·2
	Mean = 9·28	Mean = 9·16
	(s.e. ± 0·426)	(s.e. ± 0·316)

EXPERIMENT 9.

THE ACTIVITY OF CALCIUM-DEPRIVED CHICKENS IN AN ACTIVITY CAGE.

Introduction

It has been shown that rats show an increase in activity in stabilimeter cages if they are deprived of food (Campbell and Sheffield, 1953) and that a similar effect may be seen in specific deprivation of a nutrient such as thiamine (Wald and Jackson, 1944), protein (Hitchcock, 1928) or minerals in general (Spector and Young, 1957). Some specific deficiencies such as that of Vitamin D may lead to an almost immediately decrement in activity (Richter and Rice, 1943). In this present experiment an attempt was made to investigate the effect of calcium deprivation upon activity in chickens, since little work appears to have been carried out on this problem in birds.

Subjects

These were 6 seven-week-old broilers.

Materials and Methods

Activity was recorded in a cylindrical cage of diameter 36 ins. and height 22 ins. The floor was mounted on a central pivot with four microswitches spaced equally around the periphery so that they were actuated whenever there was a weight shift on the floor of the cage. Each time a microswitch was actuated it advanced the total on a digital counter. Food and water were available in the cage.

Each bird was placed in the activity cage for a period of one hour at the same time each day, and its activity was considered to be proportional to the number of times the microswitches were

actuated. In point of fact, any gross movement of the bird was recorded, but small movements of the head, neck or wings did not actuate the counters. Activity was recorded for a period of 7 days when the birds were seven weeks old. The three experimental birds were then given the calcium deficient diet (Diet 2) to eat, while the controls received a high calcium diet (Diet 5). After 14 days all the birds were tested for five consecutive days, and then after 27 days all were tested again for a further five days. On the final day of testing the experimental birds had been deprived of calcium for 33 days.

Results

The results for the six birds are shown below, in Table 16.

Table 16

	Deprived			Controls		
Bird No.	6073	6066	6067	6070	6077	6062
Day i	17	132	60	4	118	105
ii	35	61	101	2	42	137
iii	24	34	96	19	11	68
iv	29	17	67	8	84	103
viii	13	10	56	36	33	23
ix	21	10	34	16	24	46
x	41	4	46	63	196	15
Daily Av.	25·6	38·3	65·7	21·1	72·5	71·0
Day 14	69	44	12	15	4	38
15	32	142	48	28	8	105
16	48	165	32	81	38	71
17	19	198	8	49	0	51
18	59	72	7	74	0	114
Daily Av.	45·4	124·2	21·4	49·4	10·0	75·8

[Contd.

Table 16 (Contd.)

	Deprived			Controls		
Bird No.	6073	6066	6067	6070	6077	6062
Day 27	111	205	196	30	480	351
28	116	204	136	31	378	283
29	150	321	102	27	27	211
32	52	293	67	50	9	164
33	87	340	84	43	62	0
Daily Av.	103·2	272·6	119·0	36·2	191·2	201·8

The raw data have been given in order to show the great variations that occur within a brief period in some birds. After 14 days of calcium deprivation, two deprived birds have shown an increase in activity and one has shown a decrease, while one control has shown an increase, one a decrease and one has remained substantially the same. After 28 days, all three deprived birds have shown substantial increases, while two controls show increases and one a decrease, though not to the original level.

Discussion

Statistical analysis was not carried out because the very large day to day variation in the control birds 6077 and 6062 meant that there was no baseline with which any trend which might be present in the deprived birds could be compared. The deprived birds were in fact a good deal more consistent than the controls, and in general seemed to show an increase in activity over the period. Clearly, though, it is quite impossible to say whether or not this is due to chance while the controls exhibit such wild fluctuations. Although the cage in which testing took place was

well screened with black cloth the birds were in acoustic contact with others, and it is possible that this was a disturbing factor which should be eliminated. At the time that this work was done however access to a sound-proofed room was not possible. Until this experiment can be repeated, under more closely controlled conditions and possibly using a larger number, whether or not calcium deprivation leads to an increase in so-called "random" activity must remain an open question.

EXPERIMENT 10.1

AN INVESTIGATION OF THE ROLE OF NEED REDUCTION IN THE DEVELOPMENT OF A PREFERENCE.

Introduction

Earlier experiments (1, 2 and 4) have shown the existence of a preference for diets containing calcium among chickens which have been previously deprived of the element. These experiments also indicated that the preference developed during the period of choice and was not present in full strength at the start of the period. This suggested that the preference was a learnt one, and it seemed most probable that the ingestion of the calcium-enriched diet led to some beneficial effect, that this was rewarding and thus tended to reinforce the action. The present experiment was intended to explore this hypothesis, the argument going as follows: If need reduction is the key factor in learning in this situation, birds which have been given a period of exclusive access to the calcium-enriched diet should then show more rapid learning in the choice situation than birds which have not had this advantage. It was decided to begin by giving them a period of exclusive exposure, lasting for 24 hours, to the calcium diet.

Subjects

These were 24 eight-week-old broiler chickens.

Materials and Methods

The subjects were randomly divided into 2 groups each consisting of 12 birds, and one group was fed the calcium-deficient Diet 2 containing about 0·1% Ca, while the controls received a

breeders ration with approximately 3% Ca (Diet 5). They were fed these diets for 4 weeks and then on Day 0 every bird had two plastic boxes placed in front of it. One box contained the deficient diet that the experimental group had been receiving for the previous 29 days, while the other box contained this same diet enriched with 2% of calcium carbonate powder and with the addition of a food dye, Ponceau 2R, which coloured the diet red. These diets will be referred to as the 'D diet' and the 'ca diet' respectively. Although every bird had both diets present and visible in front of it only one diet was in fact accessible - the other being covered by a sheet of transparent perspex. The experimental group and the controls were each divided into sub-groups of six birds - one sub-group receiving access to the Ca diet only while the other sub-group could only eat the D diet. Each sub-group was further divided in that half had the Ca diet on the left hand side while half had it on the right. The procedure thus was as follows:

At 17.00 on the twenty-ninth day of deprivation all food was removed from the birds.

At 09.00 on the following day, Day 0, boxes were placed in front of all birds as detailed above and they were allowed to eat from the one box only for 24 hours.

At 09.00 on Day 1 the amount of food eaten was weighed and the total weight of box and food made up to 250 gms. again. The perspex lid was removed from the previously covered box thus making both diets equally accessible, but the position of the boxes was not

otherwise altered. At 09.00 on Day 2 and onwards the boxes were weighed, made up to 250 gms. and their position switched from left to right. The experiment ceased when records of food intakes had been taken on 9 successive days.

Results

They are given in Table 22 and in detail in Table 22A. A summary is shown below in which the birds of each group are ranked in the order of their calcium preferences.

These results were examined for significance using the Mann-Whitney U test.

C = Control bird E = Experimental bird

Ca = Bird initially exposed to the calcium diet

D = Bird initially exposed to the deficient diet.

The numbers represent calcium-diet choices during the selection period.

1. Deprived v. Control Initial exposure to Ca diet

C C C C C E E E C E E E

2 4 4 4 5 5 6 7 8 9 9 9

U = $3\frac{1}{2}$ P = ·021 Deprived birds ate more Ca than controls.

2. Deprived v. Control Initial exposure to deficient diet

C C C C C E E E C E E E

1 2 3 4 $4\frac{1}{2}$ $4\frac{1}{2}$ 6 7 7 8 8 8

U = $3\frac{1}{2}$ P = ·021 Deprived birds ate more Ca than controls

3. Deprived v. Control All birds

C C C C C C C C C E C E E E C E E E E E C E E E

1 2 2 3 4 4 4 4 $4\frac{1}{2}$ $4\frac{1}{2}$ 5 5 6 6 7 7 7 8 8 8 8 9 9 9

U = $14\frac{1}{2}$ P = ·001 Deprived birds ate more Ca than controls

4. Deprived birds Initial Ca exposure v. Initial deficient exposure

D Ca D Ca D Ca D D D Ca Ca Ca

4½ 5 6 6 7 7 8 8 8 9 9 9

U = 13 P = ·48 'Ca first' ate more Ca diet than 'deficient first'.

5. Control birds Initial Ca exposure v. Initial deficient exposure

D D Ca D D Ca Ca Ca D Ca D Ca

1 2 2 3 4 4 4 4 4½ 5 7 8

U = 11½. P = ·34 'Ca first' ate more Ca diet than 'deficient first'.

It thus appears that the initial exposure to the calcium diet as opposed to the deficient diet has no significant effect upon calcium preference thereafter.

Conclusions

It appears to be established that the deprived birds show a greater preference for the Ca diet than do the normal controls. This difference is highly significant when the groups as a whole are compared, and also reaches significance when the six deprived birds in each sub-group are compared with their controls. However, the need-reduction hypothesis would postulate that the Ca-diet-exposure group should show more rapid learning, and thereby a greater Ca-diet preference, than the deficient-diet-exposure group, and that this difference should be greater in the deprived group than in the controls. Within the deprived group the six birds initially exposed to the Ca diet did eat more of the Ca diet over the experimental period than did those birds exposed to the deficient diet, but the difference between the two groups was not significant.

This same trend was present in the control group, and to a greater degree, but again was not significant. If this tendency is a real one it may be due to the conservative nature of the chicken, in that it continues to eat that food with which it was first presented in the absence of any compelling reason to alter its habits.

It may be concluded then that this experiment does not provide any support for the prediction that 24 hours of exposure to the Ca diet should lead to an earlier development of preference for calcium. There is a non-significant trend in that direction, but it is also present in the controls.

Results

Table 22

Deprived

Bird No.	Day 0	1	2	3	4	5	6	7	8	9	Ca %	Ca No.
C 1161	Cal	C	D	C	C	D	C	C	C	D	56·1	6
C 1386	Cal	C	D	C	C	D	C	C	C	C	56·0	7
C 1390	Cal	C	C	C	C	C	C	C	C	C	70·5	9
P 1298	Cal	C	C	C	C	C	C	C	C	C	58·1	9
C 1354	Cal	C	C	D	C	D	C	D	C	D	55·5	5
C 1509	Cal	C	C	C	C	C	C	C	C	C	70·4	9
Ca %		62	59	61	72	55	61	53	71	54		
C 1352	Def	D	C/D	C	C	D	C	D	D	C	55·2	4½
P 1294	Def	C	C	C	C	C	C	C	C	D	59·5	8
C 1520	Def	C	C	C	C	C	C	D	C	D	60·9	7
C 1307	Def	C	C	C	C	C	C	C	D	C	83·5	8
C 1392	Def	D	C	C	C	C	C	C	C	C	64·5	8
C 1358	Def	C	C	C	C	C	C	D	D	D	63·4	6
Ca %		59	65	63	79	66	78	49	61	50		

[Contd.

Table 22 (Contd.)

Controls

Bird No.	Day 0	1	2	3	4	5	6	7	8	9	Ca %	Ca No.
C 1395	Cal	C	C	D	D	C	C	D	D	D	48·0	4
C 1355	Cal	C	D	C	D	D	D	C	D	C	53·0	4
C 1360	Cal	D	D	D	C	C	D	D	D	D	46·5	2
C 1391	Cal	C	D	C	D	C	D	C	D	C	55·7	5
C 1357	Cal	D	C	D	C	D	C	D	C	D	49·8	4
C 1351	Cal	C	C	C	D	C	C	C	C	C	67·7	8
Ca %		58	55	60	47	56	55	47	43	49		
207	Def	D	D	C	C	C	C	C	C	C	57·7	7
204	Def·	D	C	D	D	D	D	C	D	D	46·1	2
C 1517	Def	D	C	D	D	D	D	D	D	D	35·7	1
C 1361	Def	D	C	D	D	D	C	C	D	D	46·2	3
C 1393	Def	C	C	C/D	D	D	C	D	C	D	41·0	4½
C 1359	Def	D	D	D	C	D	C	D	C	C	52·1	4
Ca %		30	52	42	46	40	61	43	63	48		

Note

C = More of the calcium diet was consumed.

D = More of the deficient diet was consumed.

C/D = Equal quantities of both diets were consumed.

Ca % = The column represents calcium-diet consumption by birds and the row that by days, both expressed as a percentage of total intake.

Ca No. = This column represents the number of calcium-diet choices by each bird over the nine-day period.

EXPERIMENT 10.2.

Introduction

This experiment is an extension of 10.1, in which birds were given an initial exposure of 24 hours to the calcium-enriched diet. In that experiment a trend for those birds to show a greater preference for the Ca diet than birds initially exposed to the deficient diet was observed, but there was no significant difference and the trend was also observed among the controls. In this experiment the period of initial exposure was 48 hours in the expectation that if the trend was real, and not a chance one, a more pronounced preference should be exhibited.

Subjects

These were 20 eight-week-old broiler chickens.

Materials and Methods

The birds were randomly divided into 2 groups each of 10 birds and then deprived exactly as in 10.1 except that the period of deprivation was 35 days instead of 30 days. On Day 35 every bird had two plastic boxes placed in front of it. One box contained the deficient diet that the group had been receiving for the previous 34 days, while the other box contained this same diet enriched with 2% of calcium carbonate powder and with the addition of a food dye - Ponceau 2R - which coloured the diet red. The birds were divided into sub-groups of five each and given access to one of the diets only as in 10.1. Three of the birds in each sub-group had the accessible box on the left hand side and two had it on the right. Each box contained 250 gms. of food. It

was weighed after 24 hours and the weight of food made up to 250 gms. again. It was weighed again at 48 hours and made up as before and now the perspex cover was removed from the other diet. Both diets were weighed on Day 3 and each day from now on they were switched across from left to right and vice versa in order to nullify any positional preference.

Results

The relative intakes of the two diets for a period of 9 days are shown in Table 23, and in full detail in Table 23A. These results were examined for significance using the Mann-Whitney U test:

C = Control bird E = Experimental bird
Ca = Bird initially exposed to the Calcium diet
De = Bird initially exposed to the deficient diet
The numbers represent calcium choices during the selection period.

Calcium deprived v. Controls

C	C	E	C	C	C	C	E	C	E	C	C	C	E	E	E	E	E	E	E
1	1	2	4	4	5	5½	6	6	7	7	7	7	7	8	9	9	9	9	9

Here U = 14½ and P = ·01. The deprived birds ate more of the Ca diet.

Two days initial exposure to Ca diet

Calcium deprived v. Controls

C	E	C	C	C	C	E	E	E	E
1	2	4	4	5	5½	6	6	9	9

Here U = 4 and P = ·096. The deprived birds ate more of the Ca diet.

Two days initial exposure to deficient diet

Calcium deprived v. Controls

C	C	C	C	E	C	E	E	E	E
1	6	7	7	7	7	8	9	9	9

Here U = 1½ and P = ·024. The deprived birds ate more of the Ca diet.

Deprived birds

Initial exposure Ca diet v. Deficient diet

Ca	Ca	Ca	De	De	De	Ca	De	Ca	De
2	6	7	7	8	9	9	9	9	9

Here U = $7\frac{1}{2}$ and P = ·36. The group initially exposed to the deficient diet ate more of the Ca diet.

Control birds

Initial exposure Ca diet v. Deficient diet

Ca	De	Ca	Ca	Ca	Ca	De	De	De	De
1	1	4	4	5	$5\frac{1}{2}$	6	7	7	7

Here U = $4\frac{1}{2}$ and P = ·12. The group initially exposed to the deficient diet ate more of the Ca diet.

Conclusions

It appears to be established from these results that pre-exposure to a calcium-enriched diet, even for a period of as long as 48 hours, does not influence a calcium-deprived bird to select the enriched diet any more markedly in a choice situation thereafter than a bird which has been exposed to the deficient diet only. There was no significant difference between the two groups (P = ·36) and in fact the birds exposed to the deficient diet showed a rather stronger tendency to select the calcium-enriched diet.

This result is difficult to explain in the context of a need reduction hypothesis. The bird is being forced to make the 'correct' choice for 48 hours, and yet at the end of this time its performance is inferior to that of a bird which has been forced into the 'wrong' choice. One might argue that since the choice is forced the bird is not actually choosing at all, but in fact it is recognised that error-free rehearsal is an extremely efficient way of learning. At this stage this result must remain paradoxical.

Results

Table 23

Bird No.	Day 1	2	3	4	5	6	7	8	9	Ca %	Ca No.
Deprived	**Ca exposure for 2 days**										
C 2239	C	C	C	C	D	C	D	C	D	66·0	6
C 2244	D	D	D	D	D	C	D	C	D	48·6	2
C 2241	C	C	C	C	C	C	C	C	C	97·4	9
C 2250	C	C	D	C	C	C	C	C	D	64·0	7
C 2369	C	C	C	C	C	C	C	C	C	82·5	9
Ca %	63	79	65	82	62	82	64	86	57		
	Def exposure for 2 days										
C 2233	C	C	C	C	C	C	C	C	C	93·0	9
C 2376	D	C	C	C	C	C	C	C	C	80·9	8
C 2364	C	C	C	C	C	C	C	C	C	92·0	9
C 2247	D	C	D	C	C	C	C	C	C	61·6	7
C 2365	C	C	C	C	C	C	C	C	C	90·4	9
Ca %	63	79	72	86	84	89	87	90	87		
Controls	**Ca exposure for 2 days**										
C 2245	C	D	D	D	C	D	C	D	C	48·9	4
C 2238	C	D	C	D	C	D	C	C/D	C	53·0	5½
C 2370	D	D	C	D	D	D	D	D	D	44·0	1
C 2368	C	C	D	C	D	D	D	C	D	49·2	4
C 2240	C	D	D	C	D	C	C	C	D	52·7	5
Ca %	54	52	50	53	39	49	55	51	40		
	Def exposure for 2 days										
C 2237	D	C	D	C	C	C	C	C	C	58·2	7
C 2375	D	D	D	D	D	D	D	C	D	35·4	1
C 2371	D	C	C	C	C	C	C	C	D	71·6	7
C 2234	D	C	C	C	C	D	C	D	C	52·3	6
C 2251	D	C	C	C	C	C	D	C	C	59·9	7
Ca %	9	72	47	69	56	65	50	68	43		

Note: For explanation of symbols see Table 22.

EXPERIMENT 10.3.

EXPOSURE TO THE CALCIUM-SUPPLEMENTED DIET FOR A PERIOD OF 4 DAYS

Introduction

This experiment was a further extension of 10.1. The main difference was that the birds were given exclusive access to one or other of the diets for a period of 4 days before they were allowed a choice.

Subjects

20 eight-week-old broilers of both sexes.

Materials and Methods

As in 10.2 except that the period of pre-exposure was 4 days and the period of choice was 5 days (for technical reasons). In addition, boxes were reversed daily from side to side during the pre-exposure as well as during the choice period.

Results

These are shown in Table 24 and in detail in 24A.

These results were examined for significance using the Mann-Whitney test.

1. Deprived v. Control All birds

C C C E C C C E C E E C C E E E E C E E

0 0 1 1½ 2 2 2 3 3 3 4 4 4 4 4½ 5 5 5 5 5

U = 21 P < ·05 Deprived birds ate more calcium than controls.

2. Deprived v. Controls Initial exposure to calcium diet.

C C C C C E E E E E

0 1 2 2 3 4 5 5 5 5

U = 0 P = ·008 Deprived birds ate more calcium than controls.

3. Deprived v. Controls Initial exposure to deficient diet

C E C E E C E C E C

0 1½ 2 3 3 4 4 4 4½ 5

U = 13 P = 1·00 No difference between deprived and control birds.

4. Deprived birds Initial exposure calcium diet v. deficient diet.

De De De De Ca De Ca Ca Ca Ca

1½ 3 3 4 4 4½ 5 5 5 5

U = 1½ P = ·024

'Ca-diet-first' group ate more calcium than 'deficient-diet-first' group.

5. Control birds Initial exposure calcium diet v. deficient diet.

De Ca Ca Ca De Ca Ca De De De

0 0 1 2 2 2 3 4 4 5

U = 6½ P = ·26 'Deficient-diet-first' group ate more calcium than 'calcium-diet-first' group (but difference not significant).

Conclusions

In this experiment (unlike 10.1 and 10.2) the deprived birds which had been exposed initially to the Ca diet did eat more than those which were exposed to the deficient diet. This difference was not seen in the control birds, perhaps rather surprisingly the group which ate more of the calcium diet was that which had been exposed to the deficient diet first. It seems therefore from these results that a four day exposure to a calcium-supplemented diet does reinforce further ingestion of this diet by deprived birds, while exposure for only 1 or 2 days does not

provide sufficient reinforcement. Since this pattern was not seen in the controls in 10.2 or 10.3 (though it was present in 10.1) it appears unlikely that it is merely a reflection of the tendency to continue eating a familiar diet in preference to a novel one.

Table 24

Deprived	Pre-exposure to calcium diet						
Bird No.	Day 1	2	3	4	5	Ca %	Ca No.
P 1426	C	C	C	C	C	93·9	5
D 357	D	C	C	C	C	61·3	4
D 421	C	C	C	C	C	58·3	5
D 363	C	C	C	C	C	61·9	5
D 419	C	C	C	C	C	73·2	5
Ca %	65	76	71	79	68		
	Pre-exposure to deficient diet						
D 359	C	C	C	C	C/D	56·3	4½
D 416	D	D	C	C	C	70·1	3
D 367	D	C	C	C	C	60·6	4
D 356	D	D	C	C	C	61·7	3
D 362	D	D	C	D	C/D	43·6	1½
Ca %	41	55	73	65	69		
Controls	Pre-exposure to calcium diet						
D 358	C	D	D	C	C	52·4	3
D 414	D	D	D	D	D	25·2	0
D 348	C	D	D	D	D	46·6	1
P 1416	D	C	D	C	D	48·2	2
P 1402	D	C	D	C	D	44·0	2
Ca %	40	45	43	54	41		

[Contd.

Table 24 (Contd.)

Controls (Contd.) Pre-exposure to deficient diet

Bird No.	Day 1	2	3	4	5	Ca %	Ca No.
D 354	C	C	C	D	C	57·6	4
D 353	D	D	D	D	D	30·1	0
P 1425	D	C	D	C	D	52·5	2
P 351	C	C	C	C	C	70·8	5
P 1446	C	C	C	C	D	51·7	4
Ca %	52	53	64	48	47		

Note: For explanation of symbols see Table 22.

EXPERIMENT 11.1

SELECTION OF CALCIUM CARBONATE FOLLOWING THE INJECTION OF A CALCIUM SALT

Introduction

If the selection of diets containing calcium by deprived birds operates through the action of a need-reduction mechanism - an important question which arises is the exact nature of this mechanism. Is it a reduction of bone pain, a reversal of the state of anxiety which may be present in cases of hypocalcaemia, or some other factor? The following experiment would, it was hoped, throw some light on this problem by looking at the effects of an injection of calcium borogluconate which preceded the choice situation used in earlier experiments. If bone pain was the main factor involved it seems unlikely that the injection of a comparatively small quantity of calcium would lead to sufficient

calcification to abolish the beneficial effect of ingesting further calcium in one of the diets in the choice situation.

Materials and Methods

The experiment was done in four parts, all replicates, in view of the large numbers of birds necessary. At the age of 8 weeks the subjects were divided into 2 groups at random. The control group was fed the high-calcium breeders ration (Diet 5), while the deprived group was fed a low calcium mash (Diet 2). At the age of 14 weeks each group was further sub-divided into two. On the day on which the experiment began all food was removed from the troughs at 09.00. At 11.00 all the birds, both deprived and control, in the group hereafter described as "Water" were given an injection of 15 mls. of boiled water, subcutaneously, half under each wing. The other group, described as "Gluconate" were similarly injected with 15 mls. of 40% calcium borogluconate for injection. They were returned to their cages where water was available to them ad libitum, and food was once more made available at 16.00. The choice situation was exactly as described earlier - the two diets available being plain calcium deficient mash, and the same mash + 2% of precipitated calcium carbonate and sufficient Ponceau 2R to give the diet a fairly deep pink coloration. The reason for the brief period of food deprivation was to make certain that any marked short term effect - either beneficial or aversive - at the time of the injection, did not become associated with either of the diets which the bird might

be eating at the time. Thereafter, for a period of 10 days, the boxes were weighed, refilled and switched from left to right each day.

Subjects

These were broilers - a total of 68 birds in all. Calcium-deprived birds numbered 32 - of these 16 were given an injection of calcium borogluconate and 16 were given saline. The controls numbered 36 - 18 receiving calcium borogluconate and 18 receiving water. The injection of calcium borogluconate appeared to have a toxic effect and this was more pronounced in the deprived birds than in the controls. Four deprived birds died following the injection and these are not represented in the results, while in both groups (controls and deprived) the total food intake is higher in the water than in the borogluconate groups, and this difference is particularly pronounced over the first three days.

Results

These are given in Tables 25 and 25A, but may be summarised as follows, using the same criteria as in previous experiments:

Group	Ca Pref.	Ca Rej.	No Pref.	Posit.Pref.	Total
Dep. Water	12	1	3	0	16
Dep. Gluconate	4	4	5	3	16
Cont. Water	1	6	8	3	18
Cont. Gluconate	0	4	8	6	18

Using the computer program 'Conprob' one group was compared with another, calculating the probability of distributions as different as these occurring by chance.

Groups	Probability
Dep. Water v. Dep. Gluconate	0·018
Cont. Water v. Cont. Gluconate	0·568
Deprived v. Controls	0·00005
Dep. Water v. Cont. Water	0·0001
Dep. Gluconate v. Cont. Gluconate	0·150

Conclusion

As would be expected, the deprived birds as a whole when compared with the controls showed a highly significant preference for the calcium, and so did the deprived Water compared to the control Water. The deprived Water exhibited a significant preference as against the deprived Gluconate, while there was no difference between the control Water and the control Gluconate. Thus in addition to the effect (albeit transient) on appetite seen in both groups, the injection of Gluconate rather than water also significantly reduced the preference for calcium. There was a tendency for the deprived Gluconate group to show a calcium preference with relation to the control Gluconate, but this tendency was not significant. What the injection of Gluconate appeared to have done was to have delayed the onset of the calcium preference rather than to have abolished it altogether. This may be seen by noting the number of calcium choices over the last four

days of selection - expressed as a percentage of the total choices.

Deprived Water	90·6%
Deprived Gluconate	67·2%
Control Water	38·9%
Control Gluconate	48·6%

This was correlated with the change in plasma calcium levels following injection of calcium borogluconate.

Table 25

Calcium Deprived Borogluconate Injection

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
C 4742	D	C	D	D	C	C	C	D	C	C	56·9	6
C 4753	C/D	D	D	D	C	D	C	D	C	D	36·5	3
C 5089	C	D	D	C	D	D	D	C	C	C	63·7	4
C 4749	D	D	C	D	C	C	C	C	C	C	57·6	7
C 5503	D	D	D	C	C	C	C	C	C	C	71·7	7
C 5500	D	D	D	D	D	D	C	D	C	D	31·4	2
C 5496	D	D	C	D	D	D	D	D	D	C	44·0	2
C 5949	C	D	D	C/D	C	C	C	C	C	C	66·9	6½
C 5961	D	D	D	C	D	D	C	C	C	C	48·4	5
C 5965	C	C	D	D	D	D	D	D	D	D	11·7	1
C 6410	D	D	D	C	C	D	C	D	C	D	51·9	4
C 6411	C/D	D	D	D	C	D	C	C	C	C	63·1	5
C 6413	C	C	C	D	C	C	C	C	C	C	72·6	8
C 6414	D	D	C	D	C	D	C	D	C	D	46·8	4
C 6420	C/D	C/D	C/D	D	C/D	D	D	C	C	C	53·0	4½
C 6432	C/D	C/D	D	D	D	D	D	C	D	D	37·8	1½
Ca %	44	25	40	34	60	44	64	53	71	55		

[Contd.

Table 25 (Contd.)

Calcium Deprived Water Injection

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
C 4741	C	D	C	D	C	C	C	C	C	C	77·1	7
C 4750	C	D	C	D	C	C	C	C	C	C	75·9	7
C 5085	C	C	C	C	C	C	C	C	C	C	86·1	9
C 5498	D	D	D	D	D	D	D	D	D	D	0·0	0
C 5499	D	D	C	C	C	C	C	C	C	C	74·2	8
C 5509	D	C	D	C	C	C	C	C	C	C	74·0	8
C 5510	D	C	C	C	C	C	C	C	C	C	87·3	9
C 5945	C	D	C	D	C	D	C	C	C	C	65·6	6
C 5952	D	D	D	C	C	C	C	C	C	C	68·3	7
C 5954	D	C	D	C	D	C	C	C	C	C	78·4	7
C 5963	C	D	D	D	D	D	D	C	C	C	37·5	3
C 6415	C	C	C	C	C	C	C	C	C	C	78·0	9
C 6421	C/D	D	D	C	C	C	C	C	C	C	74·0	7
C 6429	C	C	C	C	C	C	C	C	C	C	90·1	9
C 6435	D	D	D	D	C	C	C	D	C	C	46·3	5
C 6439	D	C	C	C	C	D	C	C	C	C	61·3	8
Ca %	39	49	49	68	72	74	81	79	84	79		
Normal Controls						Borogluconate Injection						
C 4752	D	C	D	C	D	C	D	C	D	D	46·1	4
C 5088	D	C	D	C	C	D	C	C	D	C	51·9	6
C 5091	C/D	D	D	D	D	D	D	D	D	C	31·2	1
C 5502	C/D	D	D	C	D	C	D	C	D	C	45·7	4
C 5504	D	C	D	D	D	D	D	C	D	C	43·2	3
C 5508	C/D	D	C	D	C	C	C	C	C	D	47·2	6
C 5511	D	D	D	D	D	D	C	D	C	D	37·2	2
C 5944	C/D	C/D	C/D	C	C	C	C	D	D	C/D	72·5	5½
C 5946	C/D	D	D	C	D	C	D	C	D	C	41·8	4
C 5951	C/D	D	D	C	C	C	C	D	D	D	46·2	4
C 5953	D	C	C/D	D	D	D	C	C	C	C	49·1	5½

[Contd.

Table 25 (Contd.)

Normal Controls (Contd.) Borogluconate Injection

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
C 5958	D	C	D	C	D	D	C	D	C	D	46·0	4
C 6409	C/D	C/D	C	D	C	D	C	D	C	D	53·9	4½
C 6412	D	D	C/D	D	C	C	C	C	C	C	68·2	6½
C 6416	C/D	C/D	D	C	D	C	C	D	C	D	51·4	4½
C 6423	C/D	D	D	C	D	C	D	D	D	C	44·4	3
C 6433	C/D	D	D	D	D	C	D	C	D	D	38·0	2
C 6434	D	D	D	D	D	D	D	D	D	D	17·2	0
Ca %	15	41	33	49	42	51	49	50	47	51		

Normal Controls Water Injection

C 4740	D	D	C	D	D	D	D	D	C	D	31·5	2
C 4747	C/D	C/D	D	C	D	C	D	C	D	C	49·2	4½
C 4751	C	C/D	C	D	C	D	C	C	C	D	57·9	5½
C 5086	D	D	D	D	D	D	C	D	C	D	42·1	2
C 5491	C	C	C	D	C	D	C	D	C	D	52·1	5
C 5501	C	C	D	C	D	C	D	D	D	D	45·0	3
C 5505	D	D	D	D	D	D	D	D	D	D	29·8	0
C 5512	D	D	D	D	C	D	D	C	D	D	42·1	2
C 5947	D	C	D	D	D	D	D	D	C	D	41·3	2
C 5956	D	C/D	C/D	C/D	C	D	D	D	D	D	38·0	2½
C 5962	D	D	D	D	D	D	D	D	D	D	0·3	0
C 5964	C	D	C	C	C	C	D	C	C	D	57·7	6
C 6418	C	C	D	D	D	C	D	C	D	D	47·3	3
C 6422	D	C	D	D	C	D	C/D	D	C	C	51·6	4½
C 6424	C	C	D	D	C	D	C	D	C	C	51·1	5
C 6425	D	C	C	C	D	D	C	C	C	C	54·8	7
C 6430	D	D	D	D	D	D	D	D	D	C	39·2	1
C 6437	C	D	C	D	C	D	C	D	C	D	46·2	4
Ca %	46	45	47	35	48	40	45	41	49	39		

(Explanation of symbols on next page)

C = More of Ca diet eaten
D = More of deficient diet eaten
C/D = Equal amounts of both diets eaten
Ca % = The column shows calcium-diet consumption by birds, and the row that by days, both expressed as percentages of total intake
Ca No. = These figures similarly represent the number of calcium-diet choices - Day 1 being disregarded.

EXPERIMENT 11.2

PLASMA CALCIUM LEVELS FOLLOWING THE INJECTION OF CALCIUM BOROGLUCONATE

Introduction

The purpose of this experiment was to investigate the effect of injecting 15 ml. of calcium borogluconate into calcium-deprived birds.

Subjects

Eight broilers, eight-weeks-old.

Materials and Methods

The birds were fed Diet 2 for a period of six weeks, and then, at 09.00 on Day 1, blood samples were taken from five birds. At 11.00 all eight were injected with 15 ml. of calcium borogluconate 4%, 7·5 ml. being given subcutaneously into the body wall below each wing. As in 11.1, this procedure is apparently hazardous

in deprived birds, since on the morning of Day 2 two birds were found dead. At 09.00 blood samples were taken from the remaining six birds. Then at 09.00 on Day 3 further samples were taken from four of the birds. The other two were not sampled because of the presence of haematomas around the wing veins. The blood was centrifuged and plasma calciums were estimated using the atomic absorption spectrometer.

Results

Bird No.	Plasma Calcium Levels in mg/100 ml.		
	Base Line Day 1	24 hours Day 2	48 hours Day 3
D 7770	5·0	5·0	-
D 7765	7·8	died	-
D 7757	8·4	12·7	-
D 7766	7·8	10·6	8·0
D 7768	7·0	died	-
D 7771	-	4·0	4·0
D 7772	-	8·0	7·8
D 7758	-	6·0	6·8
Mean	7·2	7·7	6·6

Unfortunately, owing to the death of two of the birds, and to failure in obtaining blood from some of the remainder, these results are not easy to interpret. The base-line results are consistently low but the changes on Day 2 show considerable individual variation. D 7757 shows a substantial rise and there is also an appreciable increase in D 7766 but the other four birds all return fairly low levels. The results for Day 3 are very much in line with those for Day 2.

Each bird was given a dose of borogluconate which contained 50 mg. of calcium. If this amount was distributed evenly throughout the extra-cullular space of these birds (which weighed 2 - 2½ kg.) it would represent an increase of 5 mg/100 ml. in the plasma calcium level. The actual increase measured was only 0·5 mg/100 ml. Even allowing for experimental error it is clear that the calcium is not remaining in the tissue fluids, and one must assume that the calcium stores, for example, bone, are being replenished.

Conclusions

The results of this experiment, taken in conjunction with 11.1, suggest that the bird probably monitors total body calcium rather than merely the level in the extracellular fluid, which will be reflected in the plasma calcium level. It seems at least possible that the influx of calcium into the body blocks, for a few days, the reinforcing effect of calcium ingested orally, although as emphasised in 11.1 the birds are selecting calcium quite strongly from about Day 5 onwards. It is, in fact, remarkable that the effect of injecting 50 mg. of calcium is so pronounced, when the daily consumption of 100 gm. of diet supplemented with 2% calcium carbonate results in the ingestion of 800 mg. of calcium each day.

EXPERIMENT 12.

SELECTION OF CALCIUM CARBONATE WHEN BOTH CHOICE DIETS ARE NOVEL

Introduction

Although earlier experiments have shown that calcium-deprived chickens choose diets containing calcium in preference to those deficient in it, the methodology of some of those experiments can be criticised, particularly in view of the findings of Rodgers and Rozin (1966) that thiamine-deprived rats show a preference for novel diets, whether or not they contain thiamine. Rodgers (1967) reported that this preference for a novel diet could also be observed in the case of calcium-deprived rats. The situation in the experiments that I have described is not absolutely comparable for the deprived birds and the controls. The controls, which have been fed a commercial breeders ration, are presented with a choice between two maize-soya bean diets, one of which contains calcium but both of which are novel. The deprived birds, which have been fed a deficient maize-soya bean ration are presented with a choice between this diet and a novel diet which though very similar, contains calcium. It could be argued that it is impossible to state whether the birds are selecting this diet because it contains calcium or because it is novel. However, whereas the thiamine-deprived rats selected the novel diet rather than the familiar diet right from the beginning, in the case of the chickens the preference typically developed only after 1 to 2 days. Nevertheless, the following experiment was devised in order to confirm that the novelty of the calcium-enriched diet is not of primary importance

in its selection. In this experiment both deprived and control birds were faced with an analogous situation in that both diets were completely novel to both groups.

Subjects

They were 29 eight-week-old broilers.

Materials and Methods

This experiment was carried out in 2 parts but for convenience it will be regarded as a single experiment. The birds were divided into two groups at random, - one group was fed the calcium-deficient maize-soya bean diet (Diet 2), and the controls were given the commercial breeders ration with 3% calcium (Diet 5). After 35 days both groups were given a choice of diets. One was a calcium-deficient (·04% Ca) diet (Diet 3) formulated from wheat, soya bean and synthetic vitamins and minerals, while the other was the same diet with the addition of 2% calcium carbonate and sufficient Ponceau 2R to impart a pink colour. Both these diets were very different in colour, texture and presumably also taste from the maize-soya diet 2. The period of choice was 10 days.

Results

These are shown in Table 26 and in full detail in 26A. A summary is given below.

Part 1.

	Ca Pref.	Def. Pref.	Posit. Pref.	No Pref.	Total
Deprived	5	-	-	2	7
Controls	-	4	-	4	8

Part 2.

	Ca Pref.	Def. Pref.	Posit. Pref.	No. Pref.	Total
Deprived	6	-	-	1	7
Controls	-	1	1	5	7
Combined					
Deprived	11	-	-	3	14
Controls	-	5	1	9	15

'Conprob' was used to test the significance of these distributions and the probabilities found were:

Part 1 P = ·006

Part 2 P = ·008

Combined P = ·00002

Discussion

It is clear that under the conditions of this experiment the deprived birds are selecting the calcium diet, and that this selection is about as pronounced as it is when only one of the diets presented to the deprived birds is novel. The graph (Fig. 7) shows that the group as a whole is consuming approximately equal amounts of each diet on the first day, but that already by the second day the preference is building up and thereafter rises steadily - a picture very similar to that observed previously. This experiment provides support for the contention that selection of calcium is not operating through a mechanism similar to that seen in the thiamine-deprived rat. If it were, performance in this present experiment should have been inferior to that in, for example, Experiment 11.

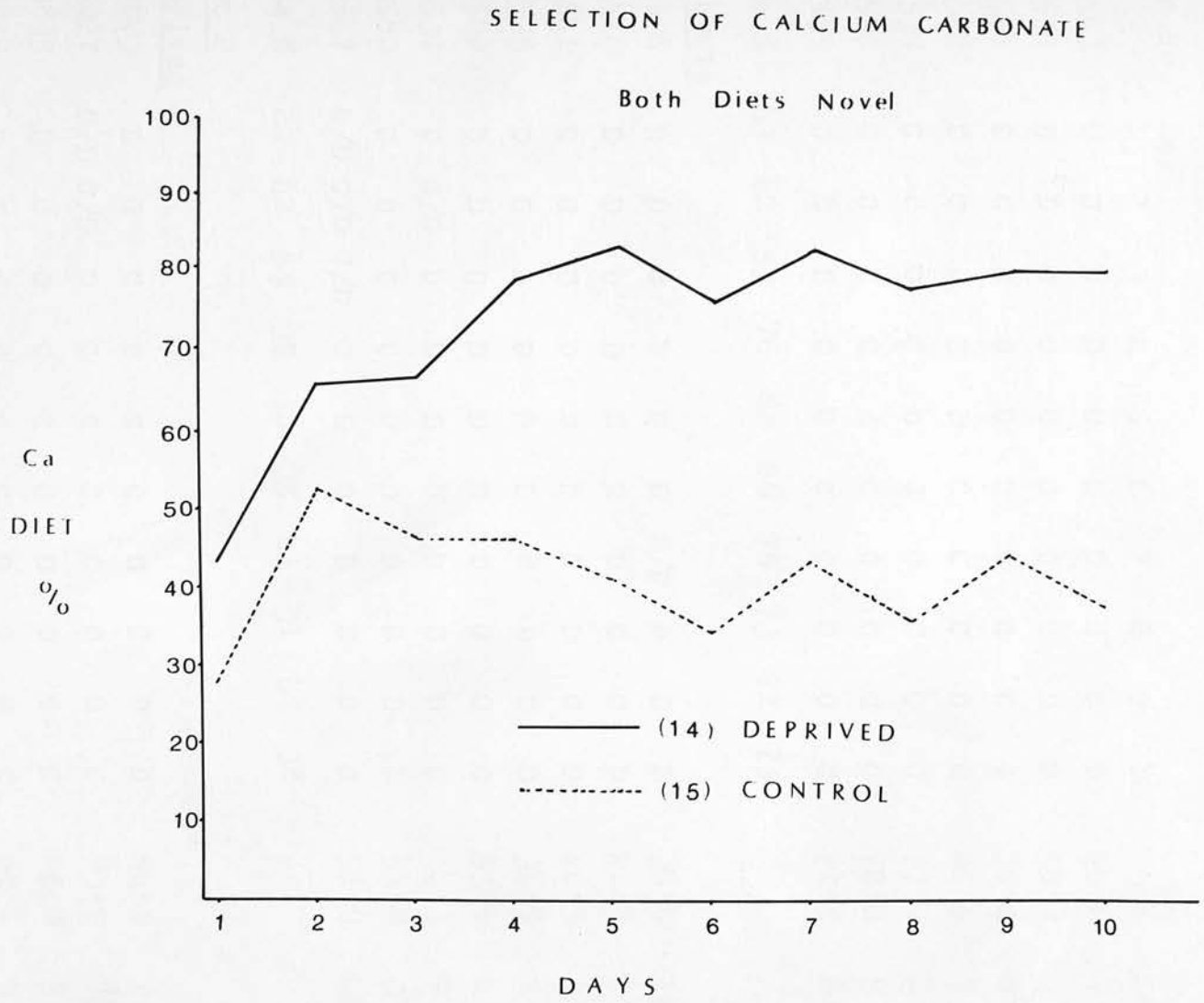


FIG. 7. The preference shown by calcium-deprived birds for a diet supplemented with 2% of calcium carbonate. Both deficient and supplemented diets were novel.

(Experiment 12)

Table 26

Part 1

Deprived

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
C 3543	D	D	D	D	C	D	D	C	C	D	34·1	3
C 3535	D	D	C	C	C	C	C	C	D	D	55·8	6
C 3551	D	C	C	C	C	C	C	C	C	C	80·4	9
C 3542	D	C	D	C	D	C	C	C	C	C	81·6	7
C 3539	C	C	D	C	C	C	C	C	C	C	77·0	8
C 3546	C	C	C	C	C	C	C	C	C	C	78·0	9
C 3549	D	C	D	C	C	C	C	C	C	C	71·7	8
Ca %	43	62	55	76	82	68	79	73	71	73		

Controls

C 3536	D	D	C	D	D	D	C/D	D	D	D	38·0	1½
C 3548	D	D	D	D	D	D	D	D	C	D	21·7	1
C 3541	D	C	D	D	D	D	C	D	C	D	40·3	3
C 3538	D	C	D	D	D	D	C	D	D	D	34·5	2
C 3540	D	D	C	D	D	D	D	C	C	C	53·6	4
C 3537	D	C/D	D	D	D	D	D	D	D	D	4·3	0
C 3550	D	C	C	C	D	D	D	D	D	D	53·9	3
C 3547	C/D	C/D	C/D	C	D	C	D	D	D	C	52·0	4½
Ca %	22	42	53	39	38	35	41	31	43	38		

Part 2

Deprived

C 3823	D	C	C	C	C	C	C	C	C	C	94·9	9
C 3827	C/D	C/D	D	C	C	C	C	C	C	C	93·0	7½
C 3835	D	C	C	C	C	C	C	C	C	C	65·0	9
C 3822	D	C	C	C	C	C	C	C	C	C	88·6	9
C 3828	C	C	C	C	C	C	C	C	C	C	96·4	9
C 3836	C	C	C	C	C	C	C	C	C	C	97·9	9
C 3821	D	D	D	D	C	D	C	C	C	C	50·4	5
Ca %	44	73	77	83	86	84	95	86	90	92		

[Contd.

Table 26 (Contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
Part 2 (Contd.)												
Controls												
C 3830	D	C	C/D	C	D	D	D	D	D	D	43·2	2½
C 3831	D	D	D	D	D	D	D	D	D	D	22·2	0
C 3832	D	C	D	C	C	D	C	D	C	D	53·3	5
C 3829	C	D	C	D	D	D	D	C	D	C	43·3	3
C 3834	D	C	D	C	C	D	D	C	D	D	45·3	4
C 3827	D	C	D	C	C	D	C	C	D	D	48·2	5
C 3825	D	C	D	D	D	C	C	C	C	D	44·6	5
Ca %	34	61	40	54	44	33	46	41	45	36		

Note: For an explanation of the symbols see Table 25.

EXPERIMENT 13

SELECTION OF CALCIUM CARBONATE WHEN THE FAMILIAR DIET CONTAINS CALCIUM WHILE THE NOVEL DIET IS CALCIUM DEFICIENT.

Introduction

This experiment is an extension of the previous one, and is essentially identical to one carried out by Rodgers and Rozin (1966), when they were investigating the specific appetite for thiamine in the rat. These workers found that if they fed rats diet A, which was thiamine-deficient, until they were deprived, and then offered them a choice between diet A (now supplemented with thiamine) and diet B (which was thiamine-deficient), the rats

exhibited a preference for diet B. It was concluded that the rats were demonstrating a learned aversion for the familiar diet, which was reflected as a preference for the novel diet. The previous experiment had shown that chickens still showed a preference for a calcium-supplemented diet, even though the choice might lie between two entirely novel diets, but it was not clear whether in some earlier experiments there might have been a degree of learnt aversion to the familiar deficient diet contributing to the preference observed for the more novel supplemented diet.

Subjects

These were fifteen eight-week-old broilers.

Materials and Methods

The chickens were divided at random into a group of eight experimental birds and seven controls. The experimental group were fed a maize-soya bean diet (Diet 2), which was calcium deficient, while the controls received a breeders ration containing 3% calcium (Diet 5). After 42 days both groups were given a choice of diets. One diet was the same maize-soya diet with the addition of 2% calcium carbonate while the other was a wheat-soya diet (Diet 3) containing ·04% calcium. Thus the experimental birds were offered a choice between a familiar, calcium-supplemented diet and a novel diet which was calcium-deficient. The controls were of course being offered a choice between two novel diets. The food intake of all the birds was measured for a ten day period of choice.

Results

These are given in Table 27 and in detail in Table 27A. They may be summarised as follows:

	Ca Pref.	Ca Rej.	Posit. Pref.	No Pref.
Deprived	6	1	-	1
Controls	5	-	-	2

No statistical tests were carried out on these results since it was sufficiently clear upon inspection that there was no significant difference between the two groups.

Conclusions

The results obtained in this experiment reinforce the conclusions reached in Experiment 12, namely, that a learned aversion to a familiar diet is not a factor in the selection of a calcium-supplemented diet by a deprived chicken, and in this respect the fowl appears to differ from the rat. When offered a choice the deprived chickens showed a preference from the first day for the familiar maize diet. It would appear from the preferences exhibited by the controls that the maize diet was more palatable than the wheat diet, which was paler and considerably more dusty owing to its high content of wheat flour.

Table 27

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
Deprived												
C 8271	C	C	C	C	C	C	C	C	C	C	88·5	9
C 8274	C	C	C	C	C	C	D	C/D	D	C	68·6	6½
C 8276	C	C	C	C	C	C	C	C	C	C	92·7	9

[Contd.

Table 27 (Contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
Deprived (Contd.)												
C 8300	C	C	C	C	C	C	C	C	C	C	65·7	9
C 8281	C	C	C	C	C	C	C	C	C	C	98·4	9
C 8269	D	D	C	C	C	D	C	C	C	C	66·7	7
C 8287	C	C	C	D	D	D	D	D	D	D	50·2	2
C 8289	D	C	C	C	C	C	C	C	C	C	91·5	9
Ca %	77	83	83	84	81	78	73	70	70	75		
Controls												
C 8285	C	C	C	C	C	C	C	C	C	D	72·6	8
C 8278	D	D	D	C	D	C	C	C	C	C	49·5	6
C 8293	C	C	C	D	D	C	C	C/D	D	C	63·4	5½
C 8264	D	C	C	C	C	C	C	C	C	C	71·8	9
C 8280	D	D	D	C	C	C	C	C	C	C	58·4	7
C 8294	C	C	C	C	C	C	C	C	C	C	98·0	9
C 8273	C	C	C	C	C	C	C	C	C	C	96·2	9
Ca %	52	57	65	71	70	78	82	73	72	80		

Note: For an explanation of the symbols see Table 25.

EXPERIMENT 14.1

THE SELECTION OF ETHYL ALCOHOL BY NORMAL AND BY CALCIUM-DEPRIVED CHICKENS.

Introduction

Attempts to produce analgesia in chickens by such recognised drugs as morphine and aspirin having failed, (see Experiment

15) it was decided to investigate the effects of alcohol, which in mammals has both an analgesic and a tranquillising effect. The latter effect is well recognised in chickens (Kovach, 1967).

Materials and Methods

An initial pilot study was carried out. Two eight-week-old broilers were placed in single cages and trained to drink from two water towers, placed side by side. Then one water tower was filled with tap water and the other with 10% ethyl alcohol, a concentration which is generally distasteful to chickens. Each day the amount drunk was recorded and the towers switched from left to right to reduce positional preference. After a period of 6 days the breeders ration (Diet 5) was replaced with a diet containing only 0·02% calcium (Diet 3), and they were fed this deficient diet for a period of 42 days. At the end of this time they were given calcium grit ad lib. One bird ate avidly, the other ate none at all, soafter a further 6 days she was force fed 10 g. daily. Records were taken for 16 days post deprivation.

Results

The relative amounts of alcohol drunk daily by the two birds are given in detail in Table 28 but the results may be summarised as follows:

In the initial period C 5946 drank approximately 3·5% of its intake as alcohol each day while C 5955 drank about 21%, although there were considerable day to day fluctuations.

Over the 6 week deprivation period both birds, after a fairly constant intake for the first 2 weeks, showed increases in intake to

about 15% and 60% by the end of the period.

In the post deprivation period C 5946 continued to show a rise after a transient fall, while C 5955, showed a progressive fall which had however begun before supplementation of the diet started.

Discussion

These results, though uncontrolled, do tend to suggest that as the chickens become more highly stressed with increasing deprivation of calcium they also show an increased voluntary consumption of 10% alcohol. However alcohol consumption is clearly controlled by factors other than calcium deprivation in view of the facts that:

a. The initial preference levels of the two birds were very different.

b. C 5946 did not show a return to baseline levels after the deficiency of calcium was restored.

c. The fall to baseline levels (and below) which occurred in C 5955 in the post deprivation period had actually begun prior to the restoration of calcium to the diet.

Table 28

Alcohol consumption as a percentage in a simultaneous-choice situation

Bird No. C 5955				Bird No. C 5946			
Day	Alcohol %	Day	Alcohol %	Day	Alcohol %	Day	Alcohol %
1	35·3	33	45·5	1	6·2	33	7·7
2	19·0	34	53·8	2	0·0	34	0·0
3	16·0	35	37·9	3	1·8	35	2·0
4	14·3	36	35·6	4	3·3	36	4·7

[Contd.

Table 28 (Contd.)

	Bird No. C 5955			Bird No. C 5946			
Day	Alcohol %	Day	Alcohol %	Day	Alcohol %	Day	Alcohol %
5	12·9	37	94·4	5	2·2	37	1·9
6	26·9	38	30·2	6	8·0	38	14·0
		39	76·9			39	2·5
7	28·2	40	51·4	7	0·0	40	2·1
8	29·0	41	54·7	8	2·4	41	3·0
9	17·9	42	54·8	9	4·7	42	-
10	15·3	43	64·1	10	0·0	43	18·2
11	19·7	44	97·2	11	2·9	44	11·5
12	19·7	45	11·7	12	5·1	45	3·7
13	21·0	46	20·0	13	4·3	46	8·5
14	20·9	47	35·5	14	-	47	47·9
15	17·5	48	6·7	15	-	48	2·5
16	36·7			16	6·7		
17	-	*49	43·3	17	2·6	*49	20·4
18	25·7	50	17·0	18	5·9	50	1·9
19	15·1	51	40·2	19	5·0	51	6·3
20	15·4	52	23·6	20	2·3	52	2·5
21	8·4	53	42·6	21	1·8	53	23·3
22	12·5	54	9·4	22	2·6	54	3·8
23	14·3	55	48·1	23	2·2	55	39·1
24	11·1	56	45·5	24	0·0	56	18·0
25	12·1	57	15·8	25	4·1	57	59·7
26	44·4	58	15·2	26	4·1	58	0·0
27	5·9	59	25·6	27	30·4	59	10·8
28	47·3	60	6·5	28	1·9	60	28·2
29	51·6	61	0·0	29	15·9	61	46·9
30	21·9	62	11·7	30	12·0	62	17·5
31	26·9	63	8·8	31	-	63	39·5
32	47·5	64	3·4	32	7·4	64	25·4

[Note on next page

Note to Table 28

Calcium grit, in the form of cockle shell, was first offered to the birds on day 49(*). C 5946 ate 37 g. on the first day and thereafter settled down to eating between 5 and 10 gms. each day. C 5955 had eaten only 2 gms. in all by day 55 so on that day he was force fed 20 gms. and was force fed 10 gms. daily thereafter until day 64 when the experiment ceased.

EXPERIMENT 14.2.

This was essentially a replicate of 14.1, but using a total of 8 eight-week-old broilers which were divided into 2 groups of four. They were initially given four days of familiarisation with the situation (Jan. 17-20) with tap water in both towers - this also served to check that they were not showing a preference for one tower over the other. Thereafter one tower contained 10% ethyl alcohol (absolute) and the other tap water, which were switched each day from left to right. The deprived group was fed Diet 2 containing ·03% calcium from Jan. 21 while the control group received the same diet with the addition of 3% calcium carbonate. After 7 weeks both groups were given the calcium-supplemented diet to the end of the experiment.

Results

These may be found in detail in Table 29A. A summary follows:

Table 29

Weekly Average intake of Alcohol

	Controls				Experimental			
Initial Period Jan. 17-21								
	39·8	51·7	50·0	40·9	58·5	49·4	48·2	34·5
Six successive weeks from Jan. 21								
1	2·0	8·5	23·7	10·4	11·4	8·7	12·4	26·6
2	1·3	12·8	5·9	5·6	3·3	2·2	9·1	25·4
3	2·9	16·9	0·9	2·4	28·8	4·0	6·9	3·6
4	2·5	27·8	1·6	2·7	46·3	40·2	16·8	3·5
5	22·7	44·9	2·5	1·5	29·3	40·9	21·6	2·4
6	16·4	40·1	3·7	2·2	38·2	44·0	33·6	5·1
Two weeks after calcium had been restored to the diet of the deprived birds.								
7	28·2	43·2	6·8	6·1	38·7	16·8	30·5	4·9
8	41·7	24·6	7·1	4·5	40·7	26·5	19·2	13·8

Grouped Averages

Week	Controls	Deprived
1	11·1	14·8
2	6·4	10·0
3	5·8	10·8
4	8·6	26·7
5	17·9	23·5
6	15·6	40·2
7	21·7	22·7
8	19·5	25·0

NOTE. The figures represent the intake of alcohol expressed as a percentage of total intake.

Mann-Whitney U Testing of any difference between the two groups. When this is done on the weekly averages the findings are:

Week	1	2	3	4	5	6	7	8
P =	·17	·17	·10	·06	·44	·17	·56	·24

Discussion

These results are rather difficult to interpret. Two of the controls exhibited a marked aversion to alcohol initially, which became less pronounced by week 5 and later. In the other two controls the aversion was not quite so marked at first, and became even more definite later. In general the birds which were to be deprived showed an initial aversion which was not quite so obvious as that of the controls, and in three of the four birds had become very weak by the end of the deprivation period, but in the fourth had become more pronounced. Following restoration of calcium to the diet of the deprived birds the aversion became more definite again in two of the birds, did not alter in the third and remained very obvious in the fourth. When the results for all birds were grouped together the deprived birds after remaining fairly constant for the first three weeks showed a rise in the last three, with a fall again in the post-deprivation period while the controls showed a fall in weeks 2, 3 and 4 followed by a rise which remaied constant over the final four weeks. Statistical tests failed to show a significant difference between the two groups although it approached significance very closely ($P = \cdot 06$) in week four. In every one of the eight weeks the deprived group showed less aversion to alcohol than the controls and this in-built bias may have made it more difficult to perceive a trend. If the two birds which were used in the pilot study are included in the results, the probability that the difference found is

produced by chance becomes as follows: (Mann-Whitney U test)

Week	1	2	3	4	5	6	7	8
P =	·24	·18	·09	·03	·30	·13	·45	·30

It will be noted that the difference is now significant at the 5% level in week 4.

Conclusions

In general it would appear that chickens show an aversion to 10% alcohol when offered a choice between it and water but that individual birds show considerable differences in the degree of aversion exhibited and that the aversion may vary a good deal over a period of time and can become either more or less marked. There is at the very least a suggestion that calcium deprivation leads to a weakening of this aversion and this implies that calcium-deprived birds find the ingestion of an alcohol solution to be in some way rewarding.

ATTEMPTS TO UPSET THE CALCIUM-SELECTION MECHANISM OF THE CHICKEN BY MEANS OF DRUGS

Introduction

Earlier experiments had shown that chickens would learn to select calcium-enriched diets when given a choice, - it thus follows that they must find the ingestion of calcium to be in some way rewarding. The exact nature of this reward was by no means clear. However a reversal of bone decalcification and thus a reduction in (hypothesised) bone pain might be one source of reinforcement. If this was the case calcium-deprived birds under the influence of analgesic drugs might be less efficient in selecting calcium-enriched diets than similar birds which had not been drugged. The first step therefore was to find an analgesic drug effective in birds. This was a complete failure. Aspirin was given to a 16 week old broiler, - 5 grains as an oral suspension. An hour later there had been no observable effect so a further 10 grains was given. This again had no analgesic effect as gauged by pinching the foot web, but the bird behaved as though in a hot atmosphere, erecting its feathers and panting with open beak - this presumably being the antipyretic effect of the aspirin - and as it was in obvious distress the experiment was discontinued. Morphine too was ineffective. Doses of up to 50 mg. (50 x the equivalent mammalian dose) were given without the slightest effect. Other workers (Blount, 1947) have given up to 360 mg. with only a slight narcotic effect. However a brief pilot study (Experiment 14) had suggested that whereas normal birds showed an aversion to 10% ethyl alcohol when given a choice between it and tap-water, this aversion became less marked when

the birds were deprived of calcium over a period of 6 weeks.

EXPERIMENT 15

THE EFFECT OF 10% ETHYL ALCOHOL ON THE SELECTION OF A CALCIUM-ENRICHED DIET.

Introduction

The present experiment therefore was intended to establish whether the ability of calcium-deprived birds to select a calcium-rich diet would be impaired when they were given only 10% alcohol to drink.

Materials and Methods

The experiment was carried out in two stages.

Stage 1. The subjects were 18 eight-week-old broilers. They were divided into groups of 9 birds each. One group was fed the usual calcium-deficient diet (Diet 2), and the other the normal breeders ration containing 3% calcium (Diet 5). After 6 weeks the deprived group were divided into groups of 4 (W) and 5 (A), and the controls into 5 (W) and 4 (A). The (W) groups were given tap-water to drink and the (A) groups were given 10% commercial grade absolute alcohol. After 24 hours acclimatisation to these fluids, all birds were given a choice between the calcium-deficient diet, and the same diet with the addition of 2% Ca carbonate, coloured red with the food dye Ponceau 2R. The diets were presented in plastic boxes in front of each bird, and every 24 hours were weighed and then transposed from left to right. Records of food eaten were taken for a period of 10 days.

Stage 2. This was a replicate of stage 1. The subjects were 22 eight weeks old broilers and were divided into Ca-deprived - 5 (W), 6 (A)and controls - 6 (W), 5 (A). In other respects the replicate was identical to stage 1.

Results

Detailed results are given in Tables 30 and 30A. They are summarised below. The birds were placed in the four classes of Calcium Preference, Calcium rejection, Positional Preference and No Preference according to the criteria given in Experiment 1.

		Ca Pref.	Ca Rej.	No Pref.	Posit. Pref.
Ca-Deprived	Water	7	-	2	-
	Alcohol	7	2	-	2
Controls	Water	-	2	4	5
	Alcohol	1	2	3	3

The significance of these results was assessed using the contingency test 'Conprob'.

All Deprived v. All Control	P = ·0001
Deprived Water v. Control Water	P = ·0006
Deprived Alcohol v. Control Alcohol	P = ·0497
Deprived Water v. Deprived Alcohol	P = ·150
Control Water v. Control Alcohol	P = ·912

Discussion

The deprived birds as a whole when compared with all controls exhibit a preference for the calcium-supplemented diet, and this is also observed when the water and alcohol subgroups

are separately compared. There is no significant difference between those birds receiving alcohol and those receiving water, in either the deprived group or the controls. Nevertheless, there is at least a suggestion that the birds receiving alcohol perform less well than those receiving water. When the group intakes of calcium diet are compared (Fig. 8), in general the curve for the alcohol group rises more slowly and levels out at a lower point, though the differences are not great.

Conclusion

Alcohol, at least at this concentration, does not markedly impair the ability of calcium-deprived birds to select a calcium-supplemented diet rather than a deficient one. It would be interesting to repeat this experiment using a more effective tranquilliser.

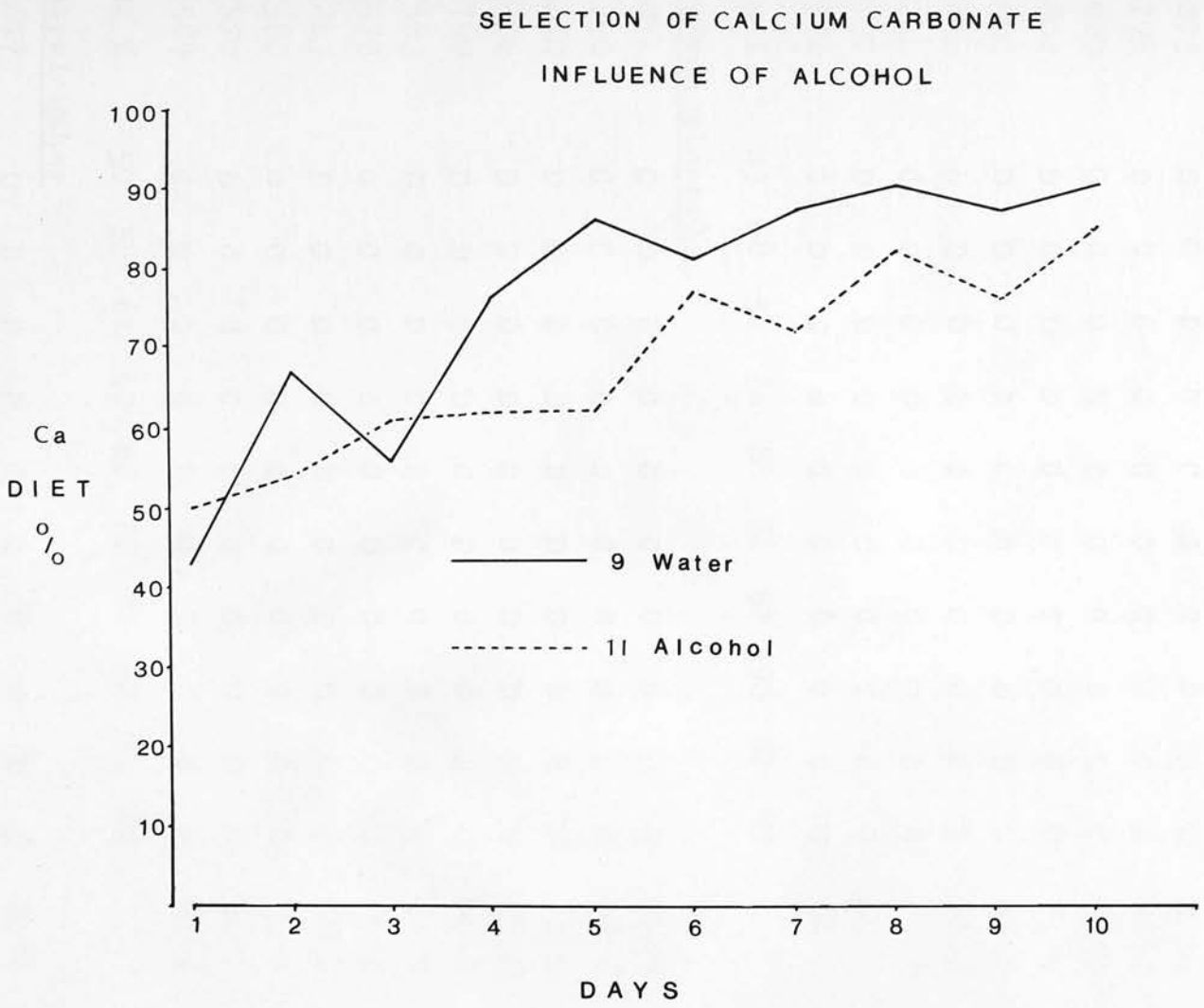


FIG. 8. The selection by calcium-deprived chickens of a diet with 2% calcium carbonate. Birds had either water or 10% ethyl alcohol to drink. (Experiment 15)

Table 30

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
Deprived Water												
C 6803	D	C	D	C	C	C	C	C	C	C	80·4	8
C 6795	D	C	C	C	C	C	C	C	C	C	90·0	9
C 6810	C	C	C	C	C	C	C	C	C	C	90·5	9
C 6802	D	C	D	C	C	C	C	C	C	C	71·4	8
C 7592	D	D	C	D	C	D	D	D	D	C	44·7	3
C 7234	D	D	D	D	C	D	C	C	C	C	56·3	5
C 7244	C	C	C	C	C	C	C	C	C	C	89·9	9
C 7237	D	D	D	C	C	C	C	C	C	C	64·4	7
C 7572	C	D	C	C	C	C	C	C	C	C	88·0	8
Ca %	43	68	56	76	86	81	87	90	87	90		
Deprived Alcohol												
C 6811	C	D	C	C	C	C	C	C	C	C	74·9	8
C 6798	C	C	C	C	C	C	C	C	C	C	77·1	9
C 6812	D	D	D	D	D	D	D	D	D	D	4·0	0
C 6806	D	D	D	D	C	D	D	D	D	C	27·3	2
C 6799	D	D	C	D	C	D	C	C	C	C	60·4	6
C 7583	D	C	D	C	D	C	C	C	C	C	71·9	7
C 7578	C	C	C	C	C	C	C	C	C	C	96·4	9
C 7243	D	C	D	C	D	C	D	C	C	C	58·5	6
C 7580	C	C	C	C	C	C	C	C	C	C	93·2	9
C 7570	D	C	C	C	C	C	C	C	D	C	58·2	9
C 7589	C	D	C	C	D	C	C	C	C	C	81·7	7
Ca %	50	54	61	62	62	78	72	82	76	85		
Control Water												
C 6817	D	D	D	D	C	D	C	D	D	D	34·7	3
C 6807	C	D	D	D	D	D	D	D	D	D	1·0	0
C 6804	C/D	C/D	C	D	D	D	D	D	D	D	16·5	1
C 6801	C	C	D	C	D	C	D	C	D	C	53·1	5
C 6797	D	C	D	C	D	C	D	C	D	C	46·7	5

Note:
For an explanation of the symbols see Table 25.

[Contd.

Table 30 (Contd.)

Control Water (Contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.
C 7588	C	D	D	D	C	D	C	C/D	C	C	51·1	4½
C 7576	C	D	C	D	C	C	C	D	C	C	58·7	6
C 7574	D	D	D	C	D	C	D	C	D	C	48·0	4
C 7585	C	D	D	D	D	C	D	C	D	C	34·5	3
C 7235	C	D	C	D	C	D	C	D	C	D	46·4	4
C 7242	D	D	D	C	D	C	C	C	D	D	46·4	5
Ca %	53	34	38	35	43	41	43	45	41	53		
Control Alcohol												
C 6808	C/D	D	D	D	C	C	C	C	D	C	45·8	5
C 6795	D	D	C	D	C	D	C	D	C	D	41·6	4
C 6185	D	D	D	D	C	D	C	D	C	D	42·4	3
C 6800	D	D	D	D	C	C	C	C	D	C	46·1	5
C 7575	D	D	C	C/D	C	D	C	D	D	D	44·2	3½
C 7582	D	D	C	C	D	D	C/D	D	C	D	38·9	3½
C 7590	D	D	D	D	D	D	D	D	C	D	35·3	1
C 7579	D	C	C	C	C	C	C	C	C	C	67·3	9
C 7587	D	D	D	D	D	D	D	D	D	D	11·3	0
Ca %	19	25	44	38	55	39	52	45	54	34		

Note: For an explanation of the symbols see Table 25.

EXPERIMENT 16

THE SELECTION OF CALCIUM CARBONATE BY CALCIUM-DEFICIENT CHICKENS UNDER THE INFLUENCE OF A TRANQUILLISER

Introduction

The findings of Experiment 15 suggested that the calcium-selection ability of birds given only 10% ethyl alcohol to drink was not markedly impaired. This present experiment examined the effects of testing birds under the influence of a tranquilliser. The drug used was S.U. 9064, available in limited quantities by courtesy of CIBA, and one of the very few tranquillisers found to be effective in the fowl (Duncan, 1969). It should not possess any marked analgesic properties at the dosage used.

Subjects

13 eight-week-old broilers.

Materials and Methods

This experiment was carried out in two parts. In the first seven birds were fed a low calcium diet (Diet 2) for 6 weeks, and then given a choice between the deficient diet and the same diet with the addition of 2% calcium carbonate and coloured pink with Ponceau 2R. For 24 hours before and during the period of choice they were given only S.U. 9064 solution to drink - 20 mg. per litre. A record was kept of both food and drug intake. The second part was similar except that six birds were tested, and they were given a higher concentration of tranquilliser - 30 mg. per litre. There were no controls used as the quantity of tranquilliser available was limited and this experiment was in the nature of a pilot study

in order to see whether it would be worth-while carrying out a full-scale experiment.

Results

These may be found in Table 31.1 and in detail in Table 31.1A. They are summarised below:

	Ca. Pref.	Ca. Rej.	Pos. Pref.	No Pref.
Part 1	3	1	0	3
Part 2	5	0	1	0

Each group was ranked in order according to their calcium choice and their intake of S.U. 9064, and tested for significance using the Kendall rank correlation coefficient.

	Correlation Coefficient	Probability
Part 1	-·43	P = ·12
Part 2	+·60	P = ·07

Discussion

Since the weights of these birds fell within a fairly narrow range, the dose per kg. of S.U. 9064 is closely related to the crude intake, so it is apparent that there is no relationship between the dose of S.U. 9064 ingested and any impairment of calcium selection. Indeed, in Part 2, where the birds were receiving a higher dose, the preference for calcium was very marked indeed. In this case the correlation coefficient is positive, indicating that the birds which ingested the largest amount of tranquilliser were those which exhibited the most clear-cut preference for calcium. These birds are receiving about 3 mg/kg of S.U. 9064 over the 14 hours of light each day, and this should be sufficient to maintain

them at a low level of tranquillisation (Beloff and Hsu, 1963). S.U. 9064 is a tranquilliser of the reserpine type and in some cases these can be cumulative over a period of time, but in the present case there was no subjective evidence of this.

Conclusions

These findings do not provide any evidence in support of the hypothesis that the mechanism for calcium selection can be blocked by drugs. In Part 1 there was a negative correlation between the dose of tranquilliser and the intake of calcium, but it was not significant, while in Part 2, with higher doses, the correlation was positive.

Table 31.1

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Ca %	Ca No.	S.U. 9064 Intake mg/day
Part 1													
2327	C	D	C	D	C	D	C	C	C	C	63·5	6	4·8
2336	D	C	C	D	D	C	C	C	C	D	54·8	6	3·4
2331	C	C	C	C	C	C	C	C	C	C	83·1	9	2·4
2788	C	C	C	C	C	C	C	C	C	C	91·3	9	2·1
2338	D	C	C	C	C	C	C	D	D	C	53·4	7	3·8
2424	D	D	C	D	C	D	D	D	D	C	47·1	3	5·4
1044	D	D	D	D	D	D	D	D	D	D	5·1	0	2·6
Ca %	50	55	67	48	66	56	66	56	62	64			
Part 2													
6106	D	D	C	C	C	C	C	C	C	C	83·4	8	5·5
6059	C	C	C	C	C	C	C	C	C	C	82·1	9	5·9
5467	D	D	C	C	C	C	C	C	C	C	61·6	8	4·3
6093	C	C	C	D	C	C	C	C	C	C	58·6	8	5·8
6090	D	C	D	C	D	C	D	C	D	C	38·8	5	3·9
6083	D	D	D	C	C	C	C	C	C	C	64·2	7	4·9
Ca %	50	51	56	67	68	71	69	75	72	75			

NOTE: For an explanation of symbols see Table 25.

EXPERIMENT 17.1

SELECTION OF FLUIDS CONTAINING CALCIUM BY CALCIUM-DEPRIVED CHICKENS

Introduction

A pilot study was carried out to investigate the response of deprived birds to different concentrations of calcium salts. Calcium lactate solution was tested at 1% and 2% and calcium acetate at 1%. These represent a calcium content of approximately 0·13%, 0·26% and 0·25% respectively, so that a broiler drinking 200-300 mls. per day would be ingesting about 500-750 mg. of calcium at the higher levels. The daily requirement is about 1,000 mg. daily, so it would be able to meet a substantial portion of its requirements. However, in a choice test where the calcium solution and tap-water were offered simultaneously all the birds tested rejected the calcium solutions after a very brief test period. Calcium borogluconate solution (4%) was also tested, and this was found to be rather more acceptable, so I decided to carry out a controlled experiment using this solution, which as a calcium content of 0·32%, thus allowing a daily intake of about 600-900 mg. a day.

Subjects

These were 8 eight-week-old broilers.

Materials and Methods

Four birds were deprived of calcium for a period of 5 weeks by feeding low calcium Diet 2, while the four controls received the breeders ration with 3% calcium (Diet 5). Each bird was then placed in an individual cage and given access to two drinkers, fluid

intake being recorded daily. The test was divided into five periods, each of four days (other than period 5 which was six days) - the contents of the drinkers being varied as shown:

Period	Drinker 1	Drinker 2
1	Water	Water
2	Water	Ca borogluconate (CBG) 4%
3	Quinine (·05%)	CBG
4	CBG	CBG
5	Quinine	CBG

Period 1 was an acclimatisation period to allow the birds to become used to the experimental situation. The drinkers were reversed from side to side throughout the tests.

Results

Controls

Bird No.	B 7895		B 7883		B 7892		B 7900		Overall Average	
Period	1	2	1	2	1	2	1	2	1	2
1	227	110	117	150	94	104	56	47	123	103
2	321	11	175	11	185	42	76	39	189	26
3	6	267	26	117	70	150	41	82	36	154
4	180	60	62	77	156	81	119	25	129	61
5	107	66	62	42	119	57	25	26	78	48

Deprived

	B 7897		B 7881		B 7896		B 7887			
1	122	85	126	140	3	22	206	52	114	75
2	257	6	209	15	196	95	102	9	191	41
3	142	11	209	4	125	46	72	6	137	17
4	32	24	96	46	159	20	74	30	90	40
5	92	9	55	34	137	34	45	116	82	48

For explanation see next page.

The figures shown are the daily fluid intakes in mls. from drinkers 1 and 2, each value being an average over 4 days (apart from period 5, which is for 6 days). The overall averages represent the totals for all 4 birds.

Discussion and Conclusions

The initial period with water in both drinkers was merely to allow the birds to become used to the situation. In period 2 all birds, deprived and controls, rejected the calcium solution in favour of water. The deprived birds ingested 17·7% and the controls 12·1% of their intake as the calcium solution. In period 3 all four of the controls showed a relative preference for the calcium solution (81·1%), while all four of the deprived birds showed a relative aversion (11·0%). In period 4 all birds were exposed to the calcium solution in both drinkers so that the deprived birds could experience any possible beneficial effect, and they were tested again in period 5. 3 out of the 4 control birds preferred the quinine on this occasion (38·1% calcium) and so did 3 out of the 4 deprived birds (36·9%) calcium). These results suggest that the birds are exhibiting a hedonic response to the fluids, much as suggested by Young (1967). The birds appear to select whichever fluid they find most palatable, and the deprived group does not ingest more of the calcium solution than the control group, even after a period when only calcium borogluconate was available, thus giving them every opportunity to experience any beneficial effect. It seems clear that a preference for calcium can be blocked by palatability factors.

EXPERIMENT 17.2

Introduction

It thus appeared that the fowl differed from the rat - whose ability to select calcium-rich solutions when deprived is well documented (Richter and Eckert, 1939). There seemed to be little purpose in investigating other soluble calcium salts - since the pilot study had shown calcium borogluconate to be the most palatable and yet it had been rejected under controlled conditions. However, calcium carbonate seemed to be a more promising candidate. It is virtually insoluble yet could be offered in the form of a suspension, when its taste would not be as marked as that of the soluble salts and so palatability factors would be of lesser importance.

Materials and Methods

Birds were tested singly in a battery cage. The fluids were presented in 500 ml. beakers at the sides of the cage. The bird was given a 3 day settling down period with tap-water provided in both beakers and then tested for 10 days, one beaker containing tap-water and the other a 1% suspension of precipitated calcium carbonate. Both beakers contained magnetic stirrers which revolved continuously, thus maintaining the calcium carbonate suspension. Beakers were reversed daily from side to side, and intake was also recorded daily.

Subjects

8 fourteen-week-old broilers were tested. Four had been fed a low calcium diet (Diet 2) for 6 weeks, while the four controls

had received the breeders ration with 3% calcium (Diet 5).

Results

Detailed results are given in Table 31.2A in the appendix. The daily intake of the calcium carbonate suspension is given below as a percentage of total intake.

Table 31.2

Deprived

Bird No. \ Day	1	2	3	4	5	6	7	8	9	10	Average
D 99	47·7	7·9	51·9	15·4	48·9	29·1	58·3	51·8	66·3	51·0	42·8
D 92	47·6	30·3	35·2	59·2	63·9	61·3	44·8	65·5	12·2	61·0	48·1
P 1437	69·9	67·5	69·6	80·0	63·6	66·7	60·2	61·5	82·0	81·5	70·3
C 8788	66·7	56·0	46·7	40·2	34·1	41·7	30·2	36·7	38·6	45·6	43·6

The overall average is 51·2%

Control

C 8829	17·8	36·2	17·6	34·7	38·4	25·1	31·3	31·6	36·2	29·2	29·8
C 2931	12·9	28·6	13·7	31·2	23·7	24·4	14·4	20·9	31·0	16·2	21·7
C 8920	21·7	19·7	32·7	16·7	29·0	16·7	38·8	31·1	18·8	23·8	24·9
D 2173	38·1	17·2	15·1	5·6	8·6	9·3	4·4	5·3	4·1	11·9	11·5

The overall average is 22·0%

These results were examined for significance using the Mann-Whitney test, and P = ·014.

Conclusions

The controls exhibit quite a marked aversion for the calcium carbonate suspension - the highest individual intake on any day was 38% and the lowest 4%. The figures for the individual deprived birds were much higher, ranging from 82% down to 8%. The group averages do not show any trend over the 10 day period - the deprived group vary between 40% and 60% and the controls between 19%

and 25%. Thus the deprived group do not show any absolute preference for the calcium suspension but they do show a relative preference with respect to the controls. This is an interesting result in that the chickens do appear to be operating according to Young's (1967) hedonic principle, and that the aversion point seems to be set at a higher level in the deprived birds. As is clear from the tests with calcium borogluconate, however, this tolerance does not extend to all aversive flavours.

SELECTION OF SODIUM BY SODIUM-DEPRIVED CHICKENS

Introduction

In these experiments it was intended to examine the response of birds which had been deprived of sodium to diets and solutions which were either sodium-enriched or sodium-deficient. The experiments all followed a similar pattern, with variations, the significant variables being the age of the birds, the length of the deprivation period and the level of added sodium chloride in the enriched diet or solution. In essence, however, the experiments followed the same pattern as those in which the response of calcium-deprived birds was examined.

EXPERIMENT 18.1

SELECTION OF SODIUM-ENRICHED DIET BY SODIUM-DEPRIVED CHICKENS

Introduction

Since it was understood that it would be difficult to deprive chickens of sodium in view of the excellent sodium retention of their kidneys, it was decided to use young chicks, in which body stores of sodium would be small, in a preliminary experiment.

Subjects

These were broiler chicks, initially twelve.

Materials and Methods

A diet was formulated in which the principal ingredients were maize, wheat, barley and soya bean. (The detailed formula may be found in the appendix (Diet 4). This diet contained upon analysis 39 p.p.m. of sodium whereas the sodium requirement of the young chick is regarded as being approximately 1100 p.p.m. (McWard and Scott, 1961). In other respects the diet was formulated in order to contain adequate quantities of all other nutrients necessary for maintenance and growth. The chicks were fed this diet in the brooder from the age of 4 days until they were 40 days old. Three birds had died in the intervening period. The remaining 9 birds were divided into 2 groups for reasons of space, one consisting of 4 chickens and the other of 5. These groups were treated in a similar fashion, being offered a choice between the sodium-deficient diet and the same diet with the addition of 2% NaCl. The amount consumed was recorded each day and the position of the two feeders was exchanged. The period of choice was 12 days in all.

Results

The amount of each diet consumed by the two groups is shown below:

Day	Sodium	Deficient	Sodium %	Sodium	Deficient	Sodium %
1	177	31	85·1	103	54	65·6
2	26	69	27·4	56	94	37·3
3	77	16	82·7	55	45	55·0
4	50	53	48·5	37	75	33·0
5	56	43	56·6	60	36	62·5
6	94	156	37·6	85	72	54·1
7	72	42	63·2	14	57	19·7
8	40	106	27·4	48	21	69·6
9	87	12	88·0	34	27	55·7
10	21	86	19·6	28	23	55·0
11	91	74	55·2	40	55	42·1
12	53	16	76·8	33	16	67·3

Since the subjects had been split into two groups, and the results obtained should be directly comparable, it is convenient to consider the results when they have been added together.

Day	Sodium %	Day	Sodium %
1	76·7	7	46·5
2	33·5	8	40·9
3	68·4	9	74·0
4	40·5	10	31·0
5	59·5	11	50·4
6	44·0	12	72·9

Discussion

On six out of the twelve days the sodium-enriched diet comprised more than 50% of the total food intake, which is clearly what would be expected if the two diets were being selected at

random. In addition there was no clear trend towards either an increased or a reduced sodium intake with time, the marked day-to-day fluctuations merely indicating the presence of a positional preference.

Although the level of plasma sodium was not measured in these birds (because of their small size) there seemed to be little doubt that they were suffering from a gross deficiency of sodium when they were tested, since they exhibited the classical symptoms associated with this condition (Nott and Combs, 1969), namely:

Dehydration - indicated by a red colouration of the shanks.

Extreme emaciation - they were less than half the weight of normal birds of this age.

High mortality - in addition to the three birds which died during the period of deprivation, another four birds died during the period of testing.

Adrenal hypertrophy - although no adrenals were weighed, those of the sodium deprived birds subjectively appeared to be plumper and more extensive than those of normal chickens of the same age.

Conclusions

It appears that sodium-deprived chickens of this age and under these conditions do not exhibit a preference for a high sodium diet. It must be borne in mind however that this was a pilot experiment and that there were no controls.

EXPERIMENT 18.2

SELECTION OF SODIUM CHLORIDE SOLUTION BY SODIUM-DEPRIVED CHICKENS

Introduction

This was again a pilot experiment, no controls being employed, its objective being to observe how sodium-deprived chickens responded to a solution of sodium chloride.

Subjects

These were six broiler chicks.

Materials and Methods

In exactly the same way as the chicks in the previous experiment these birds were deprived of sodium by being fed the sodium-deficient diet from the age of 4 days to 40 days. They were then moved into single cages where each was presented with 2 water towers, one containing tap-water and the other 1% sodium chloride, which were exchanged from left to right each day in order to minimise any positional preference. Their food remained the low-sodium diet. The quantity of fluid drunk from each container was noted daily for 4 days. Then for a further 4 days they were presented with 0·5% sodium chloride solution in both towers. A further period of choice followed, with tap-water in one tower and 0·5% sodium chloride solution in the other, for 13 days.

Results

Detailed results for individual birds are given in the appendix (Table 32A). The percentage of intake comprising NaCl solution for the group as a whole is shown below:

Day	NaCl sol^{n}	Day	NaCl sol^{n}
1	51·0%	10	76·9%
2	10·1	11	39·1
3	16·2	12	61·2
4	12·1	13	8·2
		14	61·7
5	17·1	15	10·4
6	47·1	16	55·0
7	9·3	17	5·7
8	61·2		
9	35·3		

Discussion

On the first day the water and the salt solution appear to be equally acceptable but then for the next three days the chickens exhibit a very strong aversion for the saline. Since this concentration of NaCl appeared to be so distasteful the birds were given a four day period of acclimatisation with a lower concentration, and thereafter were provided with a choice between 0·5% NaCl and water. However, the aversion continued, though not as extreme as before and superimposed over a powerful positional preference. (see Fig. 8.1.)

Conclusions

These findings bear out the point made in the previous pilot experiment in that there appeared to be no positive selection of sodium chloride in solution by the deprived birds - indeed, they actually seemed to show an aversion for it. However, it should be emphasised that this was again a pilot experiment without any controls.

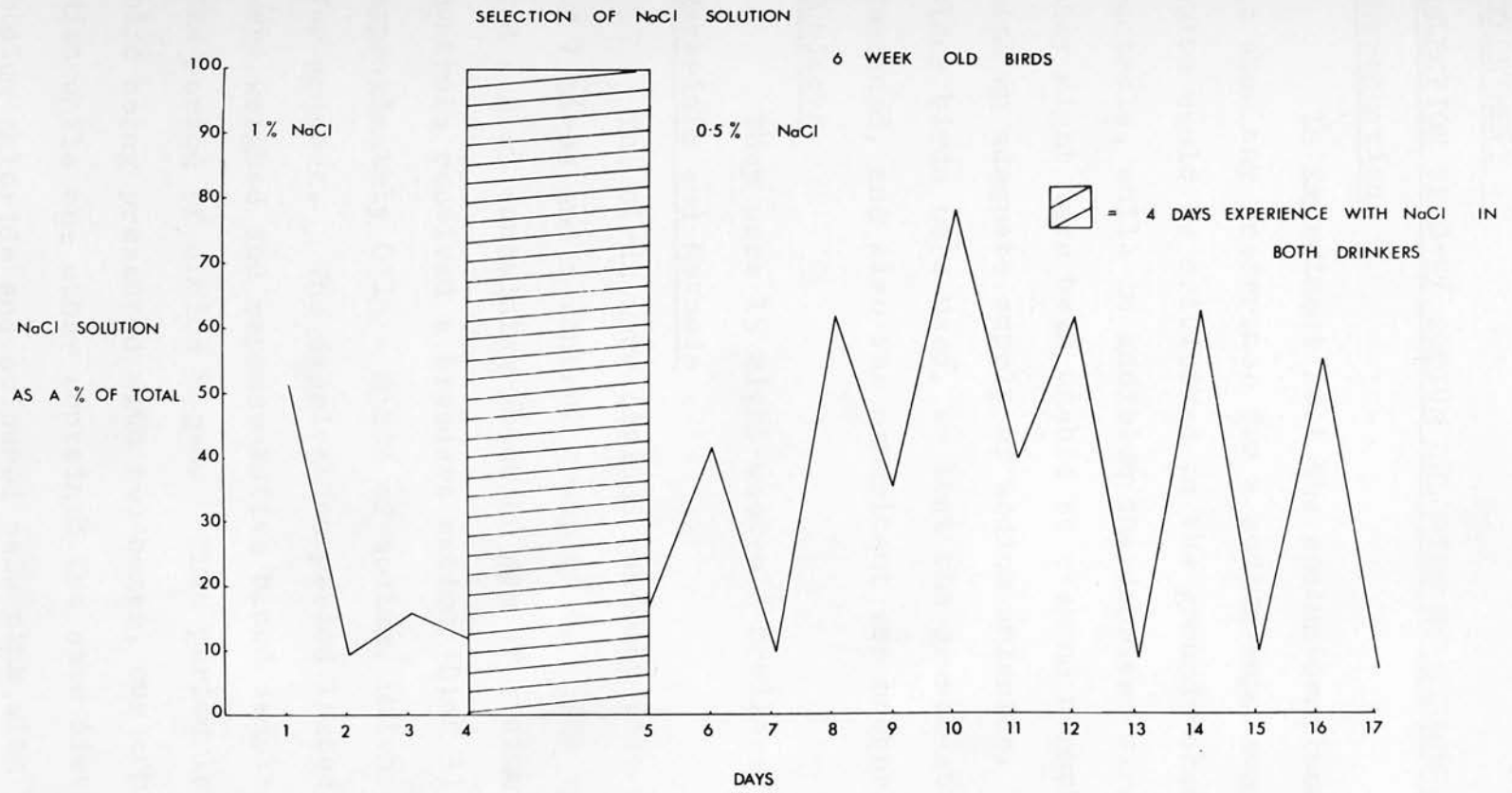


Fig. 8.1. The rejection by sodium-deprived chickens of sodium chloride solution at both 1% and 0·5% concentrations.

(Experiment 18.2)

EXPERIMENT 19

SELECTION OF 0·5% SODIUM CHLORIDE BY SODIUM-DEPRIVED CHICKENS

Introduction

In Experiment 18.1 the sodium-deprived chickens had failed to show any preference for a sodium-supplemented diet. These results could be criticised on the grounds that there were no controls, while in addition the deprived birds were so stunted that they might have been unable to respond normally even when presented with an adequate supply of sodium chloride. In this experiment older birds were used, so that the growth-stunting effect was lessened, and also the experiment was properly controlled.

Subjects

They were 15 eight-week-old broilers.

Materials and Methods

The birds were divided randomly into an experimental group of 7 birds and a control group of 8. The experimental group was fed a diet containing about 40 ppm of sodium (Diet 4) while the controls received a breeders ration (Diet 5) which contained approximately 0·15 - 0·20% of sodium, which is an adequate level for growers. The deprivation period lasted 5 weeks, and the birds were weighed and representative blood samples were taken before the period of choice began. This period lasted for 12 days, each bird being presented with two boxes, one containing the deficient diet while the other contained the same diet supplemented with 0·5% sodium chloride and coloured pale pink with the food dye Ponceau 2R. Each day the amount of food eaten from each box was recorded and

the boxes were reversed laterally in order to equalise any positional preference.

Results

The weights of the two groups are given below:

Deprived		Control	
Bird No.	Wt. (gms)	Bird No.	Wt. (gms)
D 2942	2272	D 2937	3545
D 3091	935	D 2940	2760
D 2939	1958	D 3124	2385
D 3100	1413	D 3102	2065
D 3119	1330	D 3113	3305
D 2932	1858	D 3108	2195
D 3114	1752	D 3111	3075
		D 3097	1740
Mean Wt.	1645		2634

Thus, the mean weight of the deprived group was almost 1000 g. less than that of the controls. A 't' test was carried out with d.f. = 13 and 't' = 3·296, indicating that the difference between the two groups is significant ($P < \cdot 01$).

Plasma sodium levels were also determined for 5 deprived and 5 control birds, the method used being atomic absorption spectrometry. Values obtained were as follows:

Deprived		Control	
Bird No.	Plasma Na (mg/100 mls)	Bird No.	Plasma Na (mg/100 mls)
D 2942	300	D 3652	320
D 3094	320	D 3197	320
D 3100	300	D 2933	320
D 3119	300	D 3113	340
D 2932	300	D 3111	320
Average	304		324

If these results are ranked in order of magnitude and tested for significance using the Mann-Whitney U test, the deprived birds' values are significantly lower ($P < \cdot 05$). Nevertheless, the difference is obviously a very slight one, and whether it is physiologically meaningful is open to question.

Abbreviated results of the selection of the two diets are given below in Table 33 - the full details may be found in the appendix in Table 33A.

A rapid inspection suggests that there is no major difference between the two groups, and this is borne out by applying the statistical test 'Conprob'.

	Na Pref.	Na Rej.	Pos. Pref.	No Pref.
Deprived	2	1	2	2
Control	3	1	0	4

The probability of this distribution occurring by chance is $P = 0 \cdot 658$ so one may conclude that there is no significant difference between the two groups.

Discussion

The effect of the sodium deficiency is less marked in these older birds than it was in the case of the chicks - a stunting of growth being the most obvious result. There is a fall in plasma sodium levels, but it is a very slight one. When the records of food intake are examined it is clear that the deprived group as a whole does not exhibit a preference for the supplemented diet, nor does their intake show a consistent trend over the 12 day period (Fig. 9). The intakes of individual control birds cover a

range from 21% - 71% over the test period, but in general like the deprived subjects they show neither a preference nor a rejection for the supplemented diet.

Conclusion

One may provisionally conclude that sodium-deprived chickens do not exhibit a preference for diets supplemented with approximately 0·20% sodium. This result is so surprising in view of the well documented specific appetite for sodium seen in the domestic rat that clearly this experiment should be repeated with more birds, looking also perhaps at different concentrations of sodium chloride.

Table 33

Deprived															
Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	Na %	Na No.	
D 2942	D	S	D	S	S	S	S	S	S	S	D	S	64·8	9	(8)
D 3094	D	S	D	S	S	D	S	S	S	S	S	D	67·5	8	(8)
D 2939	D	D	S	D	D	S	D	S	D	S	S	S	49·8	6	(5)
D 3100	S	D	D	D	D	S	S	S	S	S	S	D	56·3	6	(6)
D 3119	S	S	S	S	D	S	D	S	D	S	D	S	56·3	7	(6)
D 2932	S	D	S	D	S	D	S	D	S	D	S	D	45·3	5	(5)
D 3114	S	D	D	D	D	D	D	D	D	D	D	D	28·0	0	(0)
Na %	49	41	40	42	55	47	66	51	57	61	70	43			
Na No.	4	3	3	3	3	4	4	5	4	5	4	3			
Control															
D 2937	S	D	S	D	S	S	D	D	S	D	D	D	45·0	3	(3)
D 2940	D	S	S	D	S	S	S	S	D	S	D	S	55·8	8	(7)
D 3124	D	S	D	S	D	S	D	S	S	S	S	D	52·5	7	(7)
D 3102	S	S	S	S	S	S	S	D	S	S	S	S	70·7	9	(8)
D 3113	D	D	D	S	D	S	S	S	S	S	D	D	51·7	6	(6)
D 3108	D	D	D	D	D	D	S	S	S	S	D	S	35·7	4	(3)

For explanation of symbols see next page.

[Contd.

Table 33 (Contd,)

Control (Contd.)

Bird No.	1	2	3	4	5	6	7	8	9	10	11	12	Na %	Na No.	
D 3111	D	S	D	S	S	S	D	S	S	D	D	S	53·9	7	(6)
D 3097	D	D	D	D	D	D	D	D	D	D	D	S	21·4	1	(0)
Na %	35	55	34	44	51	55	52	49	54	62	45	53			
Na No.	2	4	3	4	4	6	4	5	6	5	2	5			

Note: S indicates that more of the sodium diet was consumed on that particular day, while D indicates that more of the deficient diet was eaten. SD indicates that equal quantities of the two diets were ingested.

Na % The column shows sodium-diet consumption by birds, and the row that by days, both expressed as a percentage of total intake.

Na No. These figures similarly represent the number of sodium-diet choices. The figures in brackets are corrected to refer to the first 10 days, so that they are comparable to those in most of the experiments on calcium-diet selection.

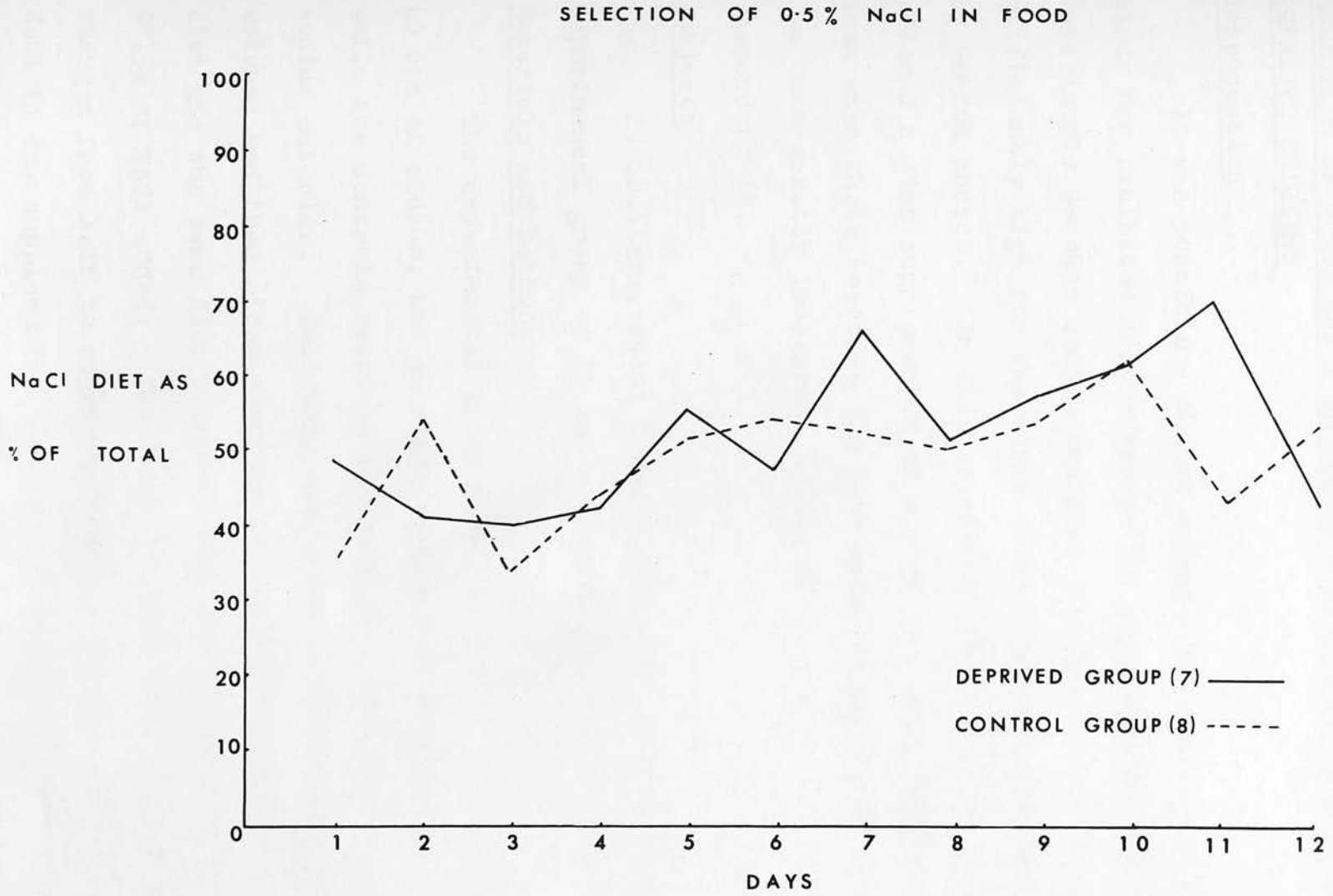


FIG. 9. The lack of preference exhibited by sodium-deprived chickens for a diet supplemented with 0'5% NaCl. (Experiment 19)

EXPERIMENT 20

SELECTION OF 0·15% and 1·5% SODIUM CHLORIDE IN FOOD BY SODIUM-DEPRIVED CHICKENS

Introduction

It was possible - though exceedingly unlikely - that deprived birds had exhibited no preference for supplemented diets in previous experiments because sodium had been incorporated at a rate sufficiently high for the birds to meet their requirements merely by random choice. In this experiment therefore the birds were offered a diet supplemented at a very low level initially, and then when their response had been established the amount of sodium was greatly increased, to see whether they continued to respond to it.

Subjects

23 broilers, about 8 weeks old, were divided into an experimental group of 11 and a control group of 12.

Materials and Methods

The experimental group were fed a diet containing about 40 ppm of sodium, but in other respects nutritionally adequate, while the controls received the same diet with the addition of 0·4% sodium chloride. Following six weeks of this regime they were weighed and then given a choice of two diets, plain low-sodium diet and the same diet coloured pink with Ponceau 2R and with 0·15% of NaCl added. The boxes of food were weighed and exchanged from left to right each day. After 4 days the amount of NaCl in the supplemented diet was increased tenfold - to 1·5% -

and intake was recorded for a further 8 days. The food dye was then switched to the deficient diet and intake was recorded for the final 7 days.

Results

The weights of the individual birds are given in detail in the appendix (Table 34A). Fourteen deprived birds had an average weight of 1·363 kg. while the fifteen controls averaged 1·579 kg. A 't' test was carried out and these means were significantly different. ($t = 2{\cdot}21$, d.f. = 17, $P < {\cdot}05$).

Details of intake of the two diets are given in Table 35 and in the appendix (Table 35A) - a summary is shown below.

Two Na numbers are given in Table 35 - the first represents the number of sodium choices in the first 12 days and the second the number in the first 10 days. In both cases Day 1 is ignored. Utilising the second number (so that it is comparable with the calcium data) the deprived and control groups were compared, using the statistical test denoted 'Conprob'.

	Na Pref.	Na Rej.	Pos. Pref.	No Pref.
Deprived	2	5	1	3
Control	1	4	2	5

The probability of these two distributions occurring by chance is 0·824.

Discussion

During the first four days, when the level of NaCl in the diet was only 0·15%, the intake of the controls varied around 50%,

and the deprived birds' intake started at about 65% but declined to approximately 50% by Day 4. There was thus no evidence of either preference or aversion in either case. When the level of added salt was increased tenfold to 1·5% both groups tended to develop an aversion to it, and this aversion was, if anything, more marked in the case of the deprived group. When the pink dye was switched to the sodium-deficient diet the intake of both deprived and control groups responded in a similar fashion, rising from around 30% to about 65%. This suggests that the aversion was a learnt one, and that the pink colour of the diet was being used as a cue. Over the final 7 days of testing the sodium-diet intake did decline slightly, but there was by no means a reversal. This suggests that the level of 1·5% NaCl was not as aversive as it had been earlier - perhaps the birds were adapting to the taste.

Conclusions

This experiment again supported the results obtained earlier in that there was still no evidence that sodium-deprived birds exhibited any preference for sodium-supplemented diets, either at very low or at very high levels. Indeed, like controls, they appeared to show an aversion at high levels.

Table 35

Sodium deprived

Bird No.	Day 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9	Na %	Na No.	
D 5422	D S D S S S D S D S D D S D S D ½ ½ D	56·5	6	(5)
D 5423	S D S D D D D D D D D D S S S D S D S	37·0	1	(1)
D 5461	D D D D D D D D D D D D S S S S S S S	8·8	0	(0)
D 5420	S S D D D D D D D D D D S D S D S D S	26·7	1	(1)

Note:
For explanation of symbols see page 246.

[Contd.

Table 35 (Contd.)

Sodium deprived (Contd.)

Bird No.	Day 1 2 3 4	5 6 7 8 9 0 1 2	3 4 5 6 7 8 9	Na %	Na No.	
D 5468	S S S D	D D S D D D D D	D S D S D S S	39·2	3	(3)
D 5449	S S S S	S S S S S S D ½	D S D S D S D	78·5	10	(9)
D 5426	S D S S	S D S S D S D D	S S S S S S S	51·1	6	(6)
D 5448	D S D S	D D D D D D D D	S D S D S D S	14·4	2	(2)
D 5444	S D D D	D D D D D D D D	S S D S D D D	17·4	0	(0)
D 5440	D S D S	D D D S D S D S	D D D D S D S	47·5	5	(4)
D 5458	S S S S	D S S S S S S S	S S S D S S S	66·0	10	(8)
Na No.	7 7 5 6	3 3 4 5 2 4 1 2½	8 7 7 5 7½5½8			

Na % (Days 1-4) 64·8 60·0 42·7 51·6

(Days 5-12) 42·9 36·7 30·0 33·4 23·3 36·5 11·9 22·9

(Days 13-19) 63·8 58·7 63·3 54·4 54·0 53·3 57·1

Control Birds

D 5459	½ S S S	S S S S S S S S	S S S D D S D	66·4	11	(9)
D 5472	D D D D	D D D D D D D D	S S S D D D D	12·0	0	(0)
D 5424	D D D D	D D D D D D D D	S D S D S D S	11·9	0	(0)
D 5436	D D D D	D D D D D D D D	S S S S S S S	1·7	0	(0)
D 5469	S S D D	D S S D S S S S	S S S S S S S	55·0	7	(5)
D 5460	D D D D	D D ½ D D D D D	S S D S D S D	22·6	½	(½)
D 5443	S S S S	S S D D D D S D	D S S S S D D	56·5	6	(5)
D 5456	D S D S	S S D S D S S S	S S S D S S D	58·5	8	(6)
D 5471	D D D ½	D S D S D S D S	S D S D S D S	43·7	4½	(3½)
D 5470	S S S S	D D D S D S D D	S D S D S D S	54·4	5	(5)
D 5466	S D S S	S D S ½-D S D D	D S D S S S S	52·5	5½	(5½)
D 5455	S D S D	S D S D S D D D	D S D S D S D	53·1	4	(4)
Na No.	5 5 5 5½	5 5 5 5 3 6 4 4	9 9 9 6 8 7 6			

Na %								
	(Days 1-4)	54·9	52·6	52·4	45·4			
	(Days 5-12)	42·3	52·7	37·0	33·4	38·0	49·5	42·2	31·6
	(Days 13-19)	65·8	54·8	62·5	46·7	52·0	60·1	51·2	

Note:
For explanation of symbols see page 246.

EXPERIMENT 21

SELECTION OF 0·7% SODIUM CHLORIDE SOLUTION BY SODIUM-DEPRIVED CHICKENS

Introduction

The response of normal fowls to NaCl solutions in the range 0·5% to 1·5% has been assessed by a number of workers. Duncan (1962) found that a marked preference was exhibited to solutions of about 0·5% and 0·6% by bantam cocks and six-week-old chicks. Kare and Ficken (1963), working with chicks, found that water and NaCl solutions were accepted indifferently at concentrations of 0·1%, 0·2% and 0·8%, while a slight preference for NaCl was exhibited at 0·4% and a slight rejection at 1·0%. However, no statistical analysis is reported and the significance of these findings is not clear. Duncan was using the method of single stimuli - Kare and Ficken do not describe their experimental procedure. Kare and Biely (1948) used a method which involved offering either water or NaCl solution on alternate days to young chicks, and they found that a slight preference was exhibited for 0·9% NaCl and a marked rejection of 1·8% NaCl. Engelmann (1934) used a simultaneous choice method and found that rejection began at 1·5%, with no preference for weak solutions. Although their methods vary to a certain extent, these workers tend to agree that fowls have a threshold of about 1·0% - 1·5%, above which they reject NaCl solutions, and below which they either show a preference or are indifferent.

The response of sodium-deprived birds to these solutions has not been examined, and this was the purpose of the present experiment.

Subjects

These were 6 fourteen-week-old broilers.

Materials and Methods

These chickens were divided into two groups of 3 birds. The experimental group was fed the sodium-deficient diet (Diet 4), while the controls received the same diet with the addition of 0·4% sodium chloride. After 6 weeks on these diets the birds were weighed and then given access to 2 drinkers, one containing tap-water and the other tap-water plus 0·7% w/v sodium chloride and in addition coloured a pale blue with methylene blue. The amount drunk from each container was recorded daily and their positions were exchanged, to minimise positional preference. Intake was recorded for 7 days, then the blue dye was transferred to the water, and records for a further 10 days were obtained.

Results

The weights of the birds at 14 weeks are given in the appendix (Table 34A), - they were part of the group described in Experiment 20 in which the sodium-deprived birds were significantly lighter than the controls. A summary of the fluid intakes is given below - the full details appear in the appendix (Table 36A). The figures represent the consumption of 0·7% NaCl, expressed as a percentage of total fluid intake.

Table 36.

Intake of NaCl solution expressed as a percentage

	Deprived				Controls			
Bird No.	D 5437	D5451	D 5446	Average Total	D 5467	D 5457	D 5478	Average Total
Day 1	51·4	2·0	0	19·8	13·3	1·8	44·6	20·4
2	3·4	6·4	3·6	4·5	63·6	32·2	25·7	41·3
3	7·4	0	67·5	32·3	5·5	2·2	14·3	6·9
4	2·8	0	2·9	1·9	92·3	2·8	14·3	36·8
5	2·4	0	10·3	3·9	8·3	5·9	13·3	9·6
6	2·2	0	9·1	3·6	47·4	5·0	14·7	20·5
7	3·4	1·7	7·7	3·5	14·6	15·8	0	11·4
Blue dye switched at this point								
8	37·5	100·0	100·0	85·3	62·1	40·9	45·2	52·0
9	27·3	100·0	10·0	27·3	15·6	14·8	29·6	19·8
10	39·0	0	68·9	43·9	69·8	12·5	60·9	59·2
11	10·9	0	32·6	19·3	83·5	6·4	65·1	64·3
12	10·4	0	3·1	9·7	5·6	7·1	54·1	23·8
13	9·8	6·9	47·9	24·6	67·8	3·3	48·4	47·1
14	40·6	5·5	8·3	19·8	4·4	5·2	2·4	3·8
15	28·9	92·1	25·5	45·5	56·1	3·9	51·7	36·7
16	10·8	86·9	6·7	25·7	2·5	4·3	4·3	3·6
17	27·6	47·8	8·6	25·3	53·8	2·8	56·6	40·6
Overall Intake	20·9	21·6	29·8	24·7	44·5	9·6	34·8	31·6

Discussion

It is quite clear from the preceding results that both deprived and control birds are rejecting the sodium chloride solution. This is true of all six birds, the aversion being least marked in the case of the control, D 5467, and most obvious in the deprived bird D 5451, during the first half of the test period. When the blue coloration was switched, the intake of saline rose sharply, indicating that the subjects had been using the colour of the solution as a cue, and it was 2, 3 or even 4 days before intake of saline had fallen back to its previously low levels (see Fig. 10). The significance of this comparatively slow reversal is very hard to assess. Clearly the taste of the saline is not highly aversive, otherwise intake would rapidly fall off, and yet it is difficult to believe that such a comparatively small amount of salt being ingested could have any aversive feedback effect, and in particular a negative feedback effect in the case of the deprived birds. There is little difference between the two groups, small as they are, although the controls cover a wider range when the overall intakes are compared.

Conclusions

Contrary to reports in the literature, normal chickens appear to exhibit an aversion to 0·7% sodium chloride when it is presented in a simultaneous-choice situation with water. This aversion is also shown by sodium-deprived birds.

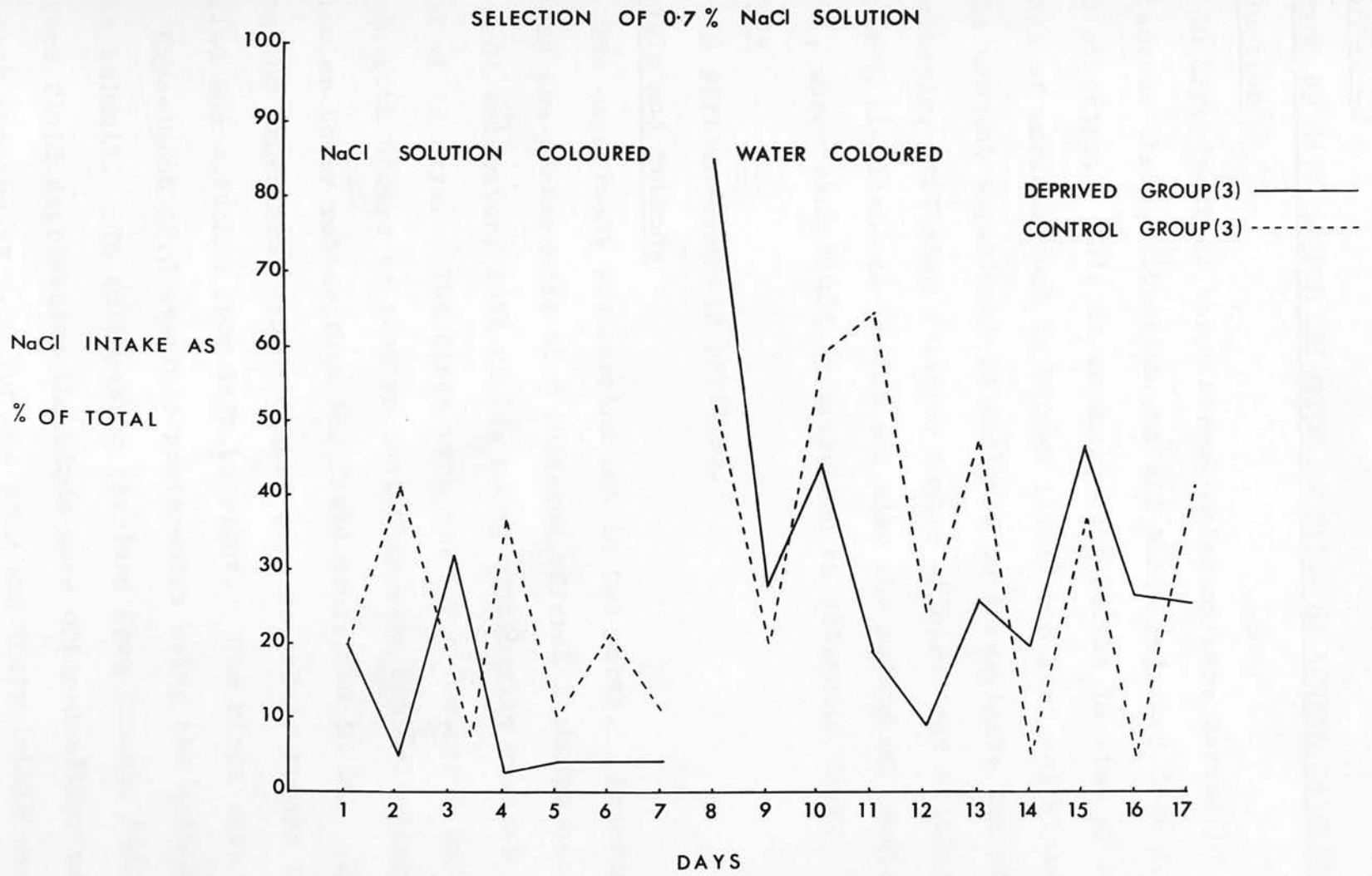


Fig. 10. Selection by sodium-deprived and normal chickens of 0.7% NaCl solution. Blue dye, acting as a cue, was switched from the NaCl to the water on Day 8.

(Experiment 21)

EXPERIMENT 22

SELECTION OF 0·7% SODIUM CHLORIDE SOLUTION BY NORMAL CHICKENS

Introduction

In Experiment 21 three normal chickens were tested in a simultaneous-choice situation and all three rejected 0·7% NaCl in favour of water. This is an unexpected result in view of the findings of workers such as Duncan (1962) and Kare and Ficken (1963), so this present experiment is designed to investigate the situation more closely, utilising a larger number of birds and testing them using both simultaneous-choice and also the method of single stimuli, where each fluid is presented on alternate days.

Subjects

16 Fifteen-week-old broilers.

Materials and Methods

The experiment was carried out in two parts. Experiment 22.1 examined the preferences of 8 chickens offered a choice between 0·7% NaCl and water, both fluids being continually present over a period of 12 days. The birds were run for a further 5 days with NaCl in both towers to confirm that they were not selecting a particular tower rather than the fluid contained in it. Each day during the experiment fluid intakes were recorded, towers freshly refilled and switched from left to right. The birds were fed Diet 5.

Experiment 22.2 examined preferences using the method of single stimuli. In this method (derived from Duncan, 1962), after 16 hours fluid deprivation the birds were offered either water or NaCl each morning at 9.30 for one hour and their intake was recorded.

They were deprived again from 10.30 to 15.30 and then given water ad lib from 15.30 to 17.30, following which they were again deprived overnight. They were tested on alternate days with 0·7% NaCl and with water, each stimulus being presented three times in all.

Results

Experiment 22.1

These are shown in Table 37.1. The group intake of sodium chloride declines from 37% on day 1 to about 20% by day 12. This indicates that the aversion was already established on the first day but that it became more pronounced with the passage of time. The average individual intakes over this period range from 40·2% down to 5·5%. All eight birds therefore are exhibiting a rejection of the NaCl, but there is considerable individual variation. When both towers contained NaCl (Days 13 - 17) intake from the tower which had previously held the NaCl was only 30·8% on Day 13 but thereafter rose to about 50%. This suggests either that this tower had become endowed with negative reinforcing properties, or that the birds had developed the habit of drinking from opposite towers on alternate days. The latter seems the more likely explanation, since the towers had no obvious distinguishing features, and chickens develop positional preferences very readily.

An interesting point is raised by Table 37.2 which shows how total fluid intake (comprising water + NaCl) goes up from a daily average of 2,764 mls. on Days 8 - 12 to 4,456 mls. on Days 13 - 17. The significance of this rise was examined using the Mann-Whitney test and the probability of it occurring by chance is P = 0·004.

Thus a relative aversion for NaCl under simultaneous-choice conditions becomes an apparent preference when NaCl is the only fluid available.

Experiment 22.2

These results are shown in Table 38. The birds consumed more NaCl on Day 2 than water on Day 1, but when Days 3 and 4, and 5 and 6 are compared they drank slightly less NaCl on both occasions. The rather large standard errors indicate that these differences are probably not real ones. Thus, whereas the birds in 22.1 exhibited an aversion for NaCl in a simultaneous choice situation, there is neither preference nor aversion when the method of single stimuli is used. However, there is a difference between the two treatments in the amount of water consumed in the two hours from 15.30 to 17.30. With each bird acting as its own control, out of 24 paired comparisons, on 22 occasions more water was consumed when it had consumed NaCl rather than water during the morning. On one occasion the same amount was consumed and on one occasion less. The Walsh test (Siegel, 1956) was used to test the significance of these findings (because measurement was on an interval scale, but, unlike the 't' test, it makes no assumptions regarding the distribution of the data).

	Day 1 v. Day 2	Day 3 v. Day 4	Day 5 v. Day 6
Probability	P = 0·09	P = 0·008	P = 0·008

Discussion and Conclusions

A number of interesting points arise from these results. In the simultaneous-choice situation, all birds exhibited an aversion

to 0·7% NaCl, confirming the tentative findings of Experiment 21, but contradicting the results obtained by Duncan (1962), Kare and Ficken (1963) and Engelmann (1934). When the same solution was tested by the method of single stimuli neither preference nor aversion could be demonstrated. This suggests that the conflicting results reported by different investigators can perhaps be accounted for in terms of their different testing procedures, and that at this borderline zone between preference, aversion and indifference, a small change in technique can have a considerable effect on acceptability. However the increased water consumption following ingestion of NaCl solution in the single stimulus test provides a possible explanation for some of these apparent contradictions. It is possible that the ingestion of the sodium chloride leads to a very slight deficit in body water, because of the need to excrete the salt load, which is manifested as thirst. This slight degree of thirst may be the negatively reinforcing factor which establishes and maintains the aversion in the simultaneous-choice situation. When the bird has no immediate choice, as in the single stimulus method, this aversion may be so slight that it does not have an observable effect. If this is the case it implies that the simultaneous-choice method is the more sensitive one.

This point, that the aversion is only seen in the presence of water, is brought out by offering only NaCl in the latter part of Experiment 22.1. The total daily intake of fluid over Days 8 - 12 is 2,764 mls. while over Days 13 - 17 it is 4,456 mls. This difference, which is highly significant, may again be related to

the increased fluid requirement in order to deal with the salt load. These results are in line with those reported by Kare and Biely (1948) where an apparent preference for 0·9% NaCl was seen when it was provided on alternate days, instead of water. They comment 'perhaps the salt water was distasteful but was balanced by the increased thirst'. It should therefore be emphasised that under certain conditions it could be fallacious to equate increased consumption with preference.

Table 37.1

Day	Bird No. 6972	6977	6755	6627	6638	6630	6988	6975	Overall Intake
1	26·0	63·1	19·3	15·8	34·6	12·3	63·8	31·7	37·0%
2	29·2	41·3	22·7	25·5	42·8	13·3	57·7	23·6	34·7%
3	48·8	32·7	13·7	22·0	28·8	10·4	50·5	40·0	34·1%
4	18·9	63·2	13·8	66·7	16·1	6·7	43·4	8·7	32·9%
5	25·7	34·0	10·6	3·4	28·6	4·7	37·0	41·2	26·0%
6	18·3	55·3	11·5	61·9	19·5	2·3	41·8	11·1	29·4%
7	30·5	26·4	8·8	24·4	41·0	6·3	26·7	28·2	25·3%
8	17·0	42·1	22·8	57·1	13·0	3·1	26·7	26·2	26·1%
9	18·8	8·5	9·4	15·5	20·8	3·9	22·2	43·9	20·6%
10	12·2	33·3	13·6	43·2	27·5	2·2	21·8	13·5	21·4%
11	38·1	11·8	5·9	21·0	14·8	0	21·6	24·0	19·7%
12	14·1	51·7	20·0	44·8	6·1	1·9	23·5	2·5	21·4%
Average	24·7	40·2	14·4	35·8	24·8	5·5	37·2	25·9	
13	62·0	49·4	8·8	10·6	43·6	32·9	21·7	31·2	30·8%
14	30·1	78·5	75·0	92·2	39·4	5·4	30·7	40·5	48·3%
15	66·2	80·0	21·7	56·2	83·3	37·2	49·2	43·1	49·6%
16	41·4	80·0	62·6	48·7	32·5	20·0	55·8	22·6	44·3%
17	59·3	86·3	26·5	21·5	50·0	86·8	27·0	64·6	52·0
Average	51·7	74·9	38·4	50·5	48·8	37·8	37·9	41·2	

For explanation see next page.

These figures represent the intake of 0·7% NaCl as a percentage from Day 1 to Day 12. On Day 13 to Day 17 both towers contained NaCl and the figures represent the intake from the tower which previously held NaCl solution.

Table 37.2

Group intake on a daily basis (mls.)

Day	Tower 1 Water	Tower 2 NaCl	Total
1	1620	950	2570
2	1610	855	2465
3	1720	890	2610
4	1935	950	2885
5	1820	640	2460
6	1725	720	2445
7	2160	730	2890
8	2075	735	2810
9	2145	555	2700
10	2055	560	2615
11	2320	570	2890
12	2205	600	2805
	NaCl	NaCl	
13	2405	1070	3475
14	2225	2080	4305
15	2375	2335	4710
16	2930	2335	5265
17	2170	2355	4525

Table 38

Intake between 9.30 and 10.30 (in ml)

Bird No.	Day 1 Water	Day 2 NaCl	Day 3 Water	Day 4 NaCl	Day 5 Water	Day 6 NaCl
D 7342	130	105	135	130	110	130
D 7344	150	210	230	155	220	180
D 7345	90	25	75	75	75	90
D 7347	110	120	95	60	150	105
D 7349	180	170	105	155	205	195
D 7350	140	170	110	120	105	120
D 7352	155	160	250	305	250	270
D 7360	135	165	140	115	150	125
Average	136·2	140·6	142·5	139·4	158·1	151·9
S.e. ±	9·1	18·8	21·1	24·8	20·4	19·7

Intake between 15.30 and 17.30 (Water) in ml

D 7342	150	150	60	135	80	150
D 7344	160	205	95	265	105	280
D 7345	145	65	65	160	165	190
D 7347	175	215	170	230	205	230
D 7349	140	200	115	165	145	190
D 7350	110	115	60	115	55	145
D 7352	205	240	240	255	215	325
D 7360	125	130	50	120	40	155
Average	151·2	165·0	106·9	180·6	126·2	208·1
S.e. ±	9·7	19·7	22·1	20·1	22·1	21·7

EXPERIMENT 23.

THE PECKING ACTIVITY OF SODIUM-DEPRIVED AND CONTROL CHICKENS AS MEASURED IN AN OPEN ARENA

Introduction

In Experiment 8 the pecking activity of a number of calcium-deprived chickens had been observed, and was shown to be significantly higher than that of controls. This present experiment was carried out on very similar lines, and was intended to determine whether sodium deprivation, like calcium deprivation, led to an increase in pecking activity.

Subjects

These were 32 eleven-week-old broilers.

Materials and Methods

The birds were divided at random into a group of 17 (experimental) and one of 15 (control), when they were 8 weeks old. The experimental group were fed Diet 4 which was low in sodium (circa 40 ppm) while the control group received the same diet with the addition of 0·5% NaCl. When this regime had continued for 22 days, the birds were removed from their home cages one at a time and placed in a six-foot-square arena for a period of 3 minutes. Facing them were four Petri dishes containing flint grit, cockle shell, perspex chips or coal chips, and all pecks directed towards any of these substances or towards the floor were scored. After testing each bird the floor was cleaned to remove droppings or chips and the next bird introduced. Deprived and control birds were tested alternately. The experimenter sat out of sight in a

hide and the birds were visually but not acoustically isolated from other fowls.

Results

The number of times each bird pecked at a particular substance or at the floor is shown in Table 39 (at the end of the experiment). A summary follows:

Number of pecks elicited by the different stimuli

Group	Perspex	Flint	Shell	Coal	Floor	Total
Deprived	0	313	487	18	314	1132
Control	0	233	0	36	111	380

Pecking activity - total number of pecks counted in individual birds.

	Mean	Range
Deprived	66·6	0 - 181
Control	25·3	0 - 78

The two groups of birds were ranked with respect to the number of pecks they gave in the three minute period and this rank order was submitted to the Mann-Whitney 'U' test. U = 61 in this case - indicating that the deprived birds pecked significantly more than the controls ($P < \cdot 02$).

Discussion

The total number of pecks given by the two groups is very different - 1132 by the deprived group of 17 birds as against 380 by the fifteen controls. The distribution is also rather different - although both groups found the perspex chips of no interest, and only two birds in each group pecked at the coal chips, eight of the deprived birds pecked at the fragments of cockle shell whilst none

of the controls did so. The pattern of preference shown by the deprived birds cannot be related to sodium content, since none of the substances provided contain an appreciable amount (the cockle shell is washed). It seems doubtful whether this difference in preference is of any significance since the pattern seen in the deprived birds is very similar to that found in the calcium-deprived birds and their controls in Experiment 8.

Conclusion

It appears from these results that sodium deprivation leads to an increase in pecking activity. A difference in the pattern of preference was also seen, but was thought to be of doubtful significance.

Table 39

Sodium Deprived Birds		Number of pecks				
Bird No.	Perspex	Flint	Shell	Coal	Floor	Total
D 5420		22	45			67
D 5422					17	17
D 5423		50	26		16	92
D 5426		60		1	36	97
D 5437						0
D 5440		53	15	17		85
D 5444		14	136			150
D 5446		16	138		27	181
D 5448		6	61			67
D 5449			65		3	68
D 5451						0
D 5458					2	2
D 5459						0
D 5461					79	79
D 5467			1		57	58
D 5468		92			3	95
D 5476					74	74
Total	0	313	487	18	314	1132

[Contd.

Table 39 (Contd.)

Control Birds

Bird No.	Perspex	Flint	Shell	Coal	Floor	Total
D 5424		43		35		78
D 5436					20	20
D 5443		7			1	8
D 5455		32			1	33
D 5456						0
D 5457		58			2	60
D 5459						0
D 5460		34				34
D 5462						0
D 5466		2		1	10	13
D 5469					64	64
D 5470						0
D 5471					13	13
D 5472		57				57
D 5479						0
Total	0	233	0	36	111	380

EXPERIMENT 24.1.

AN ATTEMPT TO PRODUCE SODIUM DEFICIENCY BY THE SUBCUTANEOUS INJECTION OF FORMALIN

Introduction

This method has been used successfully in rats to produce a functional sodium deficiency (Wolf and Steinbaum, 1965), and in this experiment a similar technique is applied in chickens, in view of the failure to produce an overt deficiency by dietary means.

Subjects

They were 10 nine-week-old broilers, weighing on average about 1·2 kg.

Materials and Methods

The subjects were randomly divided into two groups of 5 birds. The controls were injected with 12 ml. of sterile water, 6 ml. subcutaneously in the body wall on each side beneath the wing. The experimental group were similarly injected with 12 ml. of 1·5% formalin. After three hours blood samples were taken from the wing vein of all 10 birds. After 24 hours a further blood sample was taken from each of the experimental birds. The level of plasma sodium was estimated using the method of atomic absorption spectroscopy.

Results

These are given in Table 40.

Table 40

Bird No.	Plasma sodium levels in mg/100 ml.	
	3 hours	24 hours
Controls		
D 6757	360	
D 6770	340	
D 6767	400	
D 6642	400	
D 6633	400	
Mean	380 (± 11·3)	
Experimental		
D 6623	380	350
D 6763	420	350
D 6761	380	350
D 6778	420	360
D 6769	380	340
Mean	396 (± 8·8)	350 (± 2·8)

The difference between the levels at 3 hr. and at 24 hr. in the experimental birds was tested for significance using the 't' test. 't' = 4·46 with 8 degrees of freedom, and the probability of this occurring by chance is $P < \cdot 01$.

Discussion

Since there is no difference between the control and the formalin-injected birds 3 hours after the injection, but there is a significant difference between the 3 hour and the 24 hour value, it appears that plasma sodium levels may be reduced in the chicken

in the same way that they are reduced in the rat. The fall is not large, but is seen in every bird, ranging from -30 to -70 mg/ 100 ml. The average fall is one of about 12%, slightly larger than that seen in the rat (6%), when a similar dose of formalin is given - 10 ml. of 1·5% formalin per kg. body weight. Thus, one may conclude that formalin injection, unlike dietary deprivation, leads to a demonstrable fall in plasma-sodium levels, and it seems likely that the mechanism is identical to that postulated for the rat. Wolf and Steinbaum (1965) explain that the injection produces local oedema, and this is also seen in the chicken, which leads to the removal of both sodium ions and water from the general circulation. The hypovolaemia is quickly restored, and since there is no general store of sodium in the body, hyponatraemia follows.

EXPERIMENT 24.2.

SELECTION OF SODIUM CHLORIDE SOLUTION BY FUNCTIONALLY SODIUM-DEFICIENT CHICKENS

Introduction

Experiment 24.1 has shown that a functional sodium deficiency may be produced by the injection of formalin, and this experiment is intended to examine any effect that it may have on behaviour.

Subjects

They were 6 twelve-week-old broilers, their weights averaging about 2·0 kg.

Materials and Methods

These birds were fed Diet 4 (with 0·7% added NaCl) for a few days and trained to consume all their fluid from a water tower. They were then given a choice, one tower containing 0·7% NaCl solution and the other tap water. The towers were exchanged from left to right daily and the consumption noted. After 6 days on this regime they were injected with 20 ml. of 1·5% formalin, 10 ml. on each side. Their fluid consumption was recorded for a further 3 days, and over this period they were fed Diet 4 without added NaCl, so that it was sodium-deficient.

Results

The relative intake of sodium chloride is given in Table 41 at the end of the experiment and in full detail in Table 41A. A summary is given below:

Day	Overall Intake (%)	Day	Overall Intake (%)
1	29·7	7	19·4
2	41·4	8	16·0
3	29·3	9	24·7
4	29·6		
5	15·8		
6	16·5		

Discussion

In general, the intake of NaCl solution over the first six days shows the pattern seen in Experiment 22, with an irregular but progressive fall over the period. Some birds, for example D 8118, clearly find the saline more aversive than others. However, by Day 5 all the birds are exhibiting an aversion. The injection of formalin on Day 6 had remarkably little effect on saline intake, which, by analogy with the findings in rats (Wolf and Seinbaum, 1965), should have risen to a peak on Day 7 and then returned to base-line levels again on Days 8 and 9. In fact, there was a very slight overall increase on Day 7 followed by a very small fall on Day 8. A rather larger rise on Day 9 indicates however that these were probably chance fluctuations, and this is borne out by examining the records of individual birds. The only bird showing the expected pattern is D 8132, and this can be readily accounted for on the basis of a positional preference.

Conclusions

It appears therefore that, even under conditions of functional sodium deficiency, there is no preference for a sodium chloride solution rather than water.

EXPERIMENT 24.3

SELECTION OF A SODIUM-ENRICHED DIET BY FUNCTIONALLY SODIUM-DEFICIENT CHICKENS

Introduction

This experiment is essentially similar to the previous one except that the effect of formalin injection on the intake of sodium chloride in food is being examined.

Subjects

They were 6 twelve-week-old broilers, weighing on average about 2·0 kg.

Materials and Methods

As before, the initial period was spent in establishing a base-line. For six days they were given a choice between Diet 4, which was low in sodium, and the same diet with the addition of 0·5% NaCl, coloured a light red with the food dye Ponceau 2R. On Day 6 each bird was given 20 ml. of 1·5% formalin, as in the previous experiment, and food intake was recorded for a further 3 days.

Results

These are given in Table 42 at the end of the experiment. A summary is given below:

Intake of sodium-enriched diet

Day	Overall Intake (%)	Day	Overall Intake (%)
1	45·4	7	56·8
2	53·4	8	58·6
3	42·9	9	42·7
4	61·5		
5	48·5		
6	56·3		

Discussion

It will be seen from this table that there is no evidence that the formalin injection has led to a marked increase in the intake of sodium-enriched diet on the day following the injection. The overall percentage of sodium diet ingested shows virtually no change at all. D 8116 exhibits a positional preference throughout and this is not disturbed by the injection. D 8128 is the only bird which shows a rise on Day 7 followed by a fall on Day 8, but it seems most likely that this is merely a chance fluctuation.

Conclusions

When all three parts of this experiment are considered together, one must conclude that although a formalin injection produces, as in the rat, a functional sodium deficiency, there are no obvious behavioural consequences. Although this appears, at first sight, paradoxical, Wolf and Stricker (1967) point out that the elicitation of sodium appetite is multifactorial, at least in the rat, and that release of substances such as angiotensin from the kidney may play a vital role. It may therefore be that reasons need to be sought at this physiological level in order to account for these differences between chickens and rats.

Table 41

Relative intake of 0·7% NaCl solution (as a percentage)

Day	Bird No. D 8118	D 8120	D 8130	D 8126	D 8117	D 8132	Overall
1	9·5	32·3	16·2	7·3	43·5	55·9	29·7
2	7·7	20·8	63·4	63·6	28·8	40·5	41·4
3	2·9	20·5	68·3	11·3	20·7	37·0	29·3
4	5·0	15·2	35·4	53·7	27·1	17·0	29·6
5	3·6	14·5	27·4	4·7	3·3	43·1	15·8
6	2·0	6·0	27·7	38·4	10·0	5·4	16·5
7	6·5	23·6	19·0	16·9	14·8	31·9	19·4
8	24·6	18·4	26·8	6·7	10·4	4·9	16·0
9	2·5	25·0	49·2	29·7	10·9	16·7	24·7

Table 42

Intake of sodium-enriched and deficient diets (in gm)

Bird No.	Day 1	2	3	4	5	6	7	8	9
D 8119	98	78	66	81	76	99	78	64	65
	20	43	62	85	74	91	33	60	65
D 8124	41	77	126	127	80	85	71	39	83
	118	106	93	86	81	68	62	30	82
D 8136	64	75	95	84	101	64	84	99	133
	55	68	49	59	73	88	62	58	59
D 8121	51	25	36	39	76	85	68	46	41
	66	92	101	101	88	66	57	90	107
D 8128	92	87	77	110	156	86	103	79	60
	69	60	119	68	73	109	9	65	92
D 8116	1	88	1	119	3	127	3	105	0
	89	6	109	1	134	1	86	2	108
Na diet	347	430	401	560	492	546	407	432	382
Def diet	417	375	533	350	523	423	309	305	513
Total	764	805	934	910	1015	969	716	737	895
Na %	45·4	53·4	42·9	61·5	48·5	56·3	56·8	58·6	42·7

Note: The sodium-enriched diet is given first in each case.

EXPERIMENT 25

SELECTION OF THIAMINE-ENRICHED FOOD BY CHICKENS DEFICIENT IN THIAMINE

Introduction

The preference exhibited for thiamine-enriched food and liquid by thiamine-deficient rats has been well documented, but similar studies have not been carried out on chickens. In this experiment a deficiency was produced by the intramuscular injection of oxythiamine chloride hydrochloride (Sigma Chemical Co.). This technique has been used successfully in rats, where as little as 1 mg/kg will produce the symptoms of thiamine deficiency, such as nervousness and incoordination, within 12 hours. Larger doses, over longer periods, however, are required in the chicken.

Subjects

A total of 39 nine-week-old broiler chickens was used.

Material and Methods

The experiment was carried out in three parts, the first being essentially a pilot study.

Experiment 25.1.

Ten birds were randomly divided into two groups of five. The experimental birds received plain, white synthetic diet (Diet 6 in the Appendix) which was deficient in thiamine. The controls received the synthetic diet with 20 mg of thiamine added per kg of food, the diet being coloured pink with Ponceau 2R. On Day -6 the five experimental birds received 30 mg. of oxythiamine (= 20 mg/kg of body weight) in 1 ml. of water into the breast muscle, while the controls

received similarly 1 ml. of sterile water only. The same procedure was carried out on Day -4 and -2. By Day -1 the food intake of the experimental birds had sharply declined, whereas that of the controls was rising slightly. None of the nervous symptoms of thiamine deficiency was however present. Selection was begun on Day 0. Each bird was given a choice between plain, low-thiamine Diet 6, and the same diet with the addition of 20 mg. of thiamine per kg. of food, coloured pink with Ponceau 2R. Boxes were weighed daily and transferred from left to right to counter positional preferences. Records of food intake were taken for a period of 10 days.

Results

These are summarised below and given in fuller detail in Table 43 (at the end of this experiment) and 43A (in the Appendix).

	Thiamine Preference	Thiamine Rejection	Positional Preference	No Preference
Deprived	2	0	1	2
Control	1	0	0	4

Discussion

The number of birds involved is really too small to draw any definite conclusions; however, it is clear that the experimental design is poor. Both experimental and control birds tend to continue eating during the selection period that diet to which they were exposed during the six days over which the injections were given. Thus, although both groups have consumed much the same quantity of thiamine-supplemented diet over the selection period, the control group started at a high level (73%) and fell to about

50%, while the experimental group began at a low level (8%) and rose to about 50%. The results, therefore, are ambiguous, for this rise could be due to the reinforcing effect of thiamine, or alternatively to the conservative nature of the bird, which initially prefers the diet to which it is accustomed, but gradually increases its intake of a novel diet. Clearly, this experiment should be repeated, with an improved experimental design.

Experiment 25.2.

Materials and Methods

The procedure followed was similar to that in 25.1 except that both control and experimental groups received plain, unsupplemented Diet 6 during the pre-selection period. In addition, although an injection of 30 mg. of oxythiamine was given on Day -6, the amount was reduced to 15 mg. on Days -4 and -2. As before, the food intake of the experimental birds (but not the controls) was declining by Day 0, suggesting that the thiamine deficiency was becoming established. As before, records were taken over a 10 day period. There were four experimental and six control birds.

Results

These are summarised below and given in fuller detail in Tables 44 and 44A.

	Thiamine Preference	Thiamine Rejection	Positional Preference	No Preference
Deficient	3	0	0	1
Control	0	2	0	4

The percentage intake of thiamine-supplemented diet rises from 26% to about 80% over the selection period in the deficient birds, and

from 3% to about 45% in the control birds.

The significance of these results, as summarised above, was tested using 'Conprob', and P = ·07. If the Mann-Whitney test (which is more powerful when small numbers are involved), is used, U = 1½ and P = ·029. Thus the deficient birds do exhibit a significantly greater preference for the supplemented diet than the controls, and in spite of the small numbers of birds involved this result is suggestive. In the third part of this experiment a replicate is carried out using a larger number of birds in order to confirm that this is a repeatable result.

Experiment 25.3.

Materials and Methods

The procedure was identical to that adopted in 25.2. There were 19 subjects and they were randomly divided into an experimental group of 9 and a control group of 10.

Results

These are given in detail in Tables 45 and 45A, and a summary is shown below.

	Thiamine Preference	Thiamine Rejection	Positional Preference	No Preference
Deficient	8	0	0	1
Control	1	3	0	6

These results were tested for significance using 'Conprob', when P = ·002 and also using the Mann-Whitney test, when U = 10 and P = ·01. In this case the intake of the supplemented diet rises from 38% to 85% over the selection period in the deficient birds, and from 13% to about 60% in the controls. The deficient group

thus exhibit a significantly greater preference for the supplemented diet, and this is in spite of the fact that there is some evidence that a number of the controls have ingested such a small amount of supplemented diet over the early part of the selection period that by the end they have switched their preference abruptly to the supplemented diet, presumably because they are becoming deficient.

Discussion and Conclusions

These results appear to establish that a specific appetite for thiamine is demonstrable in the chicken, as in the rat. Fig. 11 is a graph of thiamine diet intake, expressed as a percentage over time. The intake of the controls starts at a very low level, and rises erratically, but only on Days 8 and 9 does it go much above 50%, and this may well be due to the appearance of deficiency in some of the controls, as explained above. In the case of the deprived birds, intake rises from about 40% on Day 1 to 85% on Day 3, and remains near this level for the remainder of the selection period. In absolute terms, therefore, the thiamine-supplemented diet is rejected on Day 1, a finding which would not be predicted in the case of the rat (Rodgers and Rozin, 1966). It thus appears that the chicken does not show a novel diet preference when thiamine-deficient, and so the mechanism of selection cannot be explained on this basis. It is interesting that the shape of the selection curve is very similar to that seen in the case of calcium selection by calcium-deprived birds.

Table 43

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Th %	Th No.
Deficient												
D 6775	D	D	D	D	D	T	D	T	D	T	39·9	3
D 6758	D	D	D	D	D	T	T	T	D	T	44·0	4
D 6774	D	T	T	T	T	T	D	T	D	T	61·5	7
D 6776	D	T	T	T	D	T	D	T	T	T	65·8	7
D 6765	D	D	D	T	T	T	D	D	D	T	47·2	4
Th %	8	48	37	79	44	79	31	74	33	68		
Controls												
D 6772	T	T	T	T	T	T	T	D	D	D	56·1	6
D 6780	D	D	D	T	D	T	T	T	T	T	63·9	6
D 6754	T	T	T	T	T	T	T	T	T	T	88·4	9
D 6642	T	D	D	T	T	T	T	T	D	T	57·6	6
D 6673	T	D	D	D	T	D	T	T	D	T	45·3	4
Th %	73	42	49	63	60	64	74	68	51	63		

Table 44

Deficient												
D 7152	D	D	D	T	D	T	D	T	T	T	59·9	5
D 7162	D	T	T/D	T	T	T	T	T	T	T	75·7	8½
D 7139	D	D	T	T	T	T	T	T	T	T	85·6	8
D 7161	T	T	T	T	T	T	T	T	D	T	69·1	8
Th %	26	44	57	93	64	87	67	97	74	90		
Controls												
D 7142	D	D	D	D	T	D	T	T	T	T	40·3	5
D 7156	D	D	D	D	D	T	T	T	T	D	35·8	4
D 7141	D	D	D	D	D	T	D	T	T	T	37·9	4
D 7147	D	D	D	D	D	D	D	D	D	D	1·1	0
D 7149	D	D	T	D	D	D	D	D	D	D	24·6	1
D 7144	D	D	T	D	D	T	T	T	T	T	50·3	6
Th %	3	7	36	9	27	40	42	56	46	42		

For explanation of symbols see next page.

Table 45

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Th %	Th No.
Deficient												
D 8292	T	T	T	T	T/D	T	T	T	T	T	68·2	8½
D 8294	D	T	T	T	T	T	T	T	T	T	86·1	9
D 8300	D	T	T	T	T	T	T	T	T	T	92·3	9
D 8291	T/D	T	T	T	T	T	T	T	T	T	93·4	9
D 8289	D	DD	T	T	T	T	T	T	T	T/D	71·3	7½
D 8123	D	D	D	D	D	D	D	T	T	T	53·1	3
D 8365	D	D	T	T	T	T	T	T	T	D	62·0	7
D 8312	D	D	T	T	T	T	T	T	D	T	75·0	7
D 8301	T	T	T	T	T	T	T	T	T	T	95·2	9
Th %	38	54	87	84	80	86	75	94	85	85		
Controls												
D 8303	D	D	D	D	D	D	D	D	T	T	28·4	2
D 8306	D	D	D	D	D	D	D	D	T	T	21·7	2
D 8314	D	D	D	D	D	D	D	D	D	D	2·8	0
D 8290	D	T	T	T	T	T	T	T	T	D	72·5	8
D 8297	D	D	T	D	T	D	T	T	T	D	53·9	5
D 8299	D	D	T	D	T	T	D	D	T	T	46·4	5
D 8293	D	T	T	T	D	T	D	D	T	D	50·5	5
D 8287	D	D	D	D	D	T	T	T	T	T	49·7	5
D 8295	D	D	D	D	T	T	T	T	D	D	46·5	4
D 8305	D	D	D	D	T	D	T	T	T	T	57·2	5
Th %	13	32	41	31	50	37	53	60	70	55		

Note: T = More of the Thiamine-supplemented diet was consumed

D = More of the Deficient diet was consumed

T/D = Equal quantities of both diets were eaten

The Thiamine No. represents the number of thiamine choices over the last nine days of the selection period

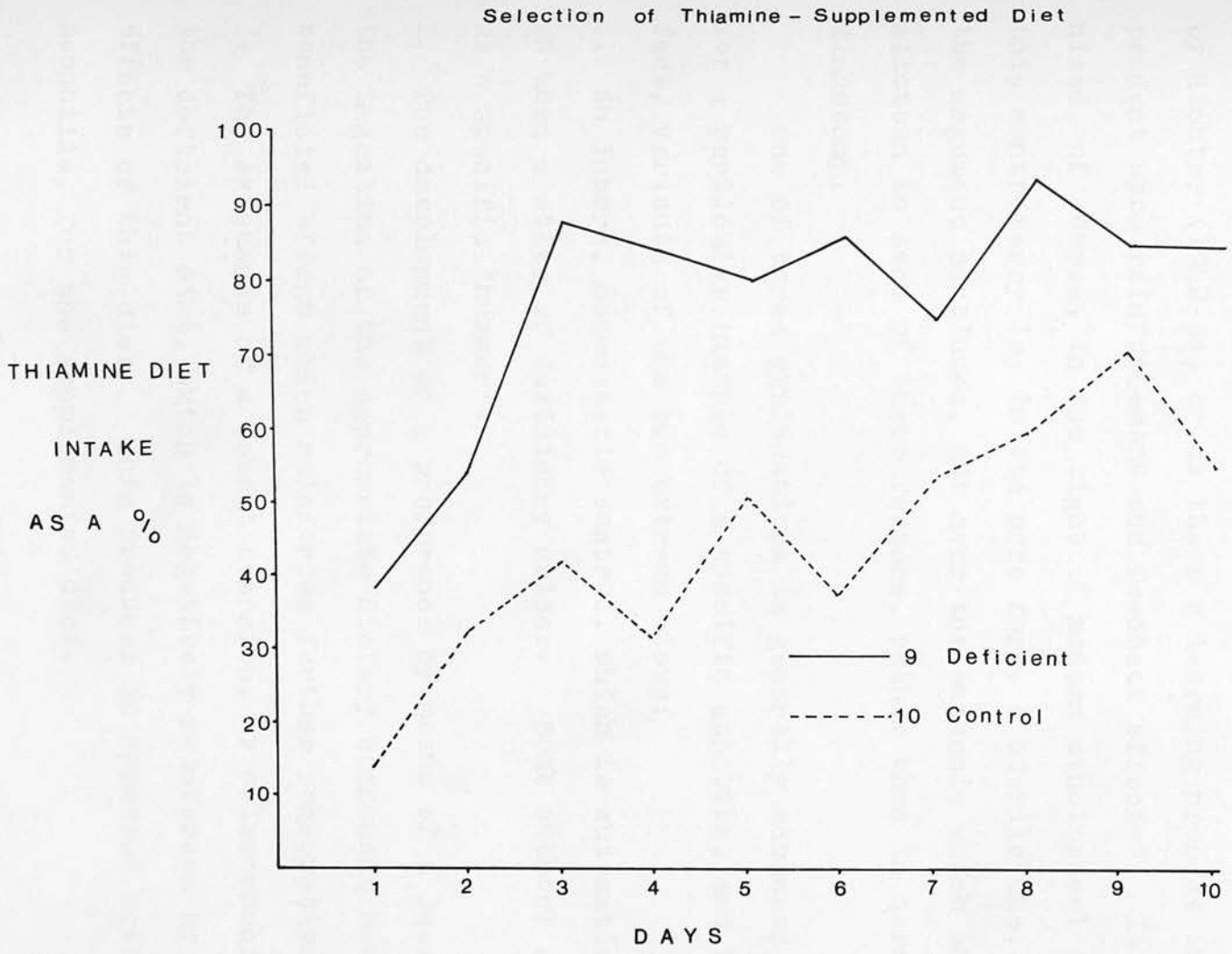


Fig. 11. Selection by thiamine-deficient and normal chickens of diet containing 20 mg thiamine hydrochloride per kg of diet.

(Experiment 25)

GENERAL DISCUSSION

The basic argument arising in a discussion on the subject of specific appetites is concerned with the mechanism which underlies them. Can the selection of an essential vitamin or mineral be explained in terms of an innate, homeostatic control, as postulated by Richter (1942-3), or is there a learning process involved, dependent upon reinforcement and feedback effects? It is now recognised, of course, in the light of modern ethological findings, that this controversy is, in its pure form, a sterile one. Nevertheless, the argument continues, but over the emphasis which should be allotted to each of these factors, rather than in terms of a pure dichotomy.

One of three explanations is generally advanced to account for a particular example of a specific appetite, and these are, in fact, variants of the two extreme views:

1. An inborn, homeostatic control, which is automatically switched on when a state of deficiency arises. Some authors describe this as a specific 'hunger'.
2. The development of a preference by means of a learning process, the ingestion of the appropriate dietary component having some beneficial effect which reinforces further consumption.
3. The existence of a learnt aversion, or palaeophobia, towards the deficient diet, which is negatively reinforced by the harmful effects of this diet. This produces an apparent preference, or neophilia, for the supplemented diet.

4. A primary neophilia, or preference for novel diets, which is triggered when an animal becomes deficient. This could be associated with exploratory behaviour.

These questions have been satisfactorily resolved in the case of the domestic rat for a number of dietary components. The selection of sodium chloride appears to be a true specific 'hunger', in that it is largely dependent upon an oropharyngeal, innate, unlearned mechanism, although there is evidence that gastric of post-ingestional feedback factors can play a part in certain circumstances. Appetites for calcium, riboflavin, nicotinic acid and pantothenic acid all seem to be mediated via some learning process, while the evidence regarding the selection of phosphorus and vitamins A and D seems to be inconclusive. At the other extreme, deficient rats appear to be indifferent to potassium, and even to actively reject magnesium.

Although the appetite for thiamine undoubtedly involves learning, Rozin and his co-workers see it in terms of a learnt aversion for the deficient diet rather than as a learnt preference for the supplemented diet, and they have attempted to apply this approach to other vitamins and minerals.

The problem of the apparent appetite for proteins and amino acids remains unresolved. Indeed, it may not be possible to talk of a specific appetite in this context at all, since the mechanism may be dependent upon the direct non-specific appetite-stimulating effects of the limiting amino acids upon that part of the brain responsible for the regulation of food intake. On the other hand it may be possible to view this effect in the context of reinforcement, in

which case it may yield an explanation in terms of cerebral events at the physiological level.

In the past there has been a tendency to concentrate exclusively on the domestic rat and to derive theoretical models of motivation which may be of general applicability, utilising only the findings which have been obtained with this species. However, the results described in this study of specific appetites in the chicken do suggest that findings obtained in one species should not be generalised to other species.

Chickens which have been deprived of calcium will generally, if given the choice, select a food which has been supplemented with a calcium salt, rather than one which is calcium-deficient. It has been argued earlier in this thesis that this is a learnt preference, and the main points will be summarised again here.

On the first day of choice the deprived group typically show an intake split 50:50 between the deficient and the supplemented diets, as do the controls. Under an unlearned homeostatic control one would expect an immediate preference. As well as a preference for calcium carbonate, which is a natural salt, chickens exhibit a preference for calcium lactate, a synthetic salt, the consumption of which would lie totally outside avian experience. When diets are matched for appearance and for taste, by incorporating calcium carbonate into one and strontium carbonate into the other, deprived birds show no selection in favour of calcium, presumably because there are no discriminable cues, with which any beneficial effect produced by the diet can be linked. Strontium carbonate as such is

not selected by calcium-deprived birds. This again militates against an explanation of the homeostatic type - a useful analogy being provided by sodium deficiency in rats. Lithium chloride has a flavour similar to sodium chloride and is selected just as freely initially, though it is rejected immediately the toxic feedback effects become apparent. If calcium selection in birds could be explained in terms of a homeostatic mechanism, strongium carbonate should be selected as freely as calcium carbonate, since its properties closely mimic those of calcium carbonate in the short term, and thus birds should select it initially. The fact that they do not, emphasises the importance of post-ingestional factors. The significant effects of these factors, which reinforce calcium selection, must be rather long-term ones, for the similarity between these two elements is considerable, at even the physiological level. Rapidly growing young chicks, which have a very high calcium requirement, can incorporate strontium to the extent of 10% in bone, when calcium is in limited supply (Weber, Doberenz, Wyckoff and Reid, 1968). Of course, a much smaller proportion (1·5%) was incorporated in the bones of the deficient birds tested in Experiment 5.2. It is interesting that strongium carbonate <u>is</u> selected by calcium-deprived rats, and there is a suggestion that a learning explanation is inadequate to explain the calcium preference of rats, since there is evidence that they do select arbitrary novel diets when calcium-deficient (Rodgers, 1967).

As to the importance of cues for the selection of a calcium-supplemented diet by chickens - selection still occurs when the diets

differ only in taste, and also when they differ only in appearance, though there is a suggestion that in both cases it is less efficient than when both cues are present.

The behaviour of calcium-deprived chickens selecting a diet supplemented with a calcium salt can thus be most readily explained in terms of a learnt preference, and it is interesting to speculate on the way in which the kind of learning seen in a two choice situation can be fitted into the framework of modern learning theories. Orthodox Hullian theory (Lunzer, 1968) can deal with this level of learning very well. Vitamin (let us say) deprivation leads to a deficit and a need, and thence to a vitamin drive. There are two stimuli presented to the animal: the vitamin enriched diet (S_v) and the deficient diet (S_d). It can make two responses: R_v and R_d, that is, ingesting the enriched or the deficient diet respectively. Superimposed on the vitamin drive is ordinary hunger drive.

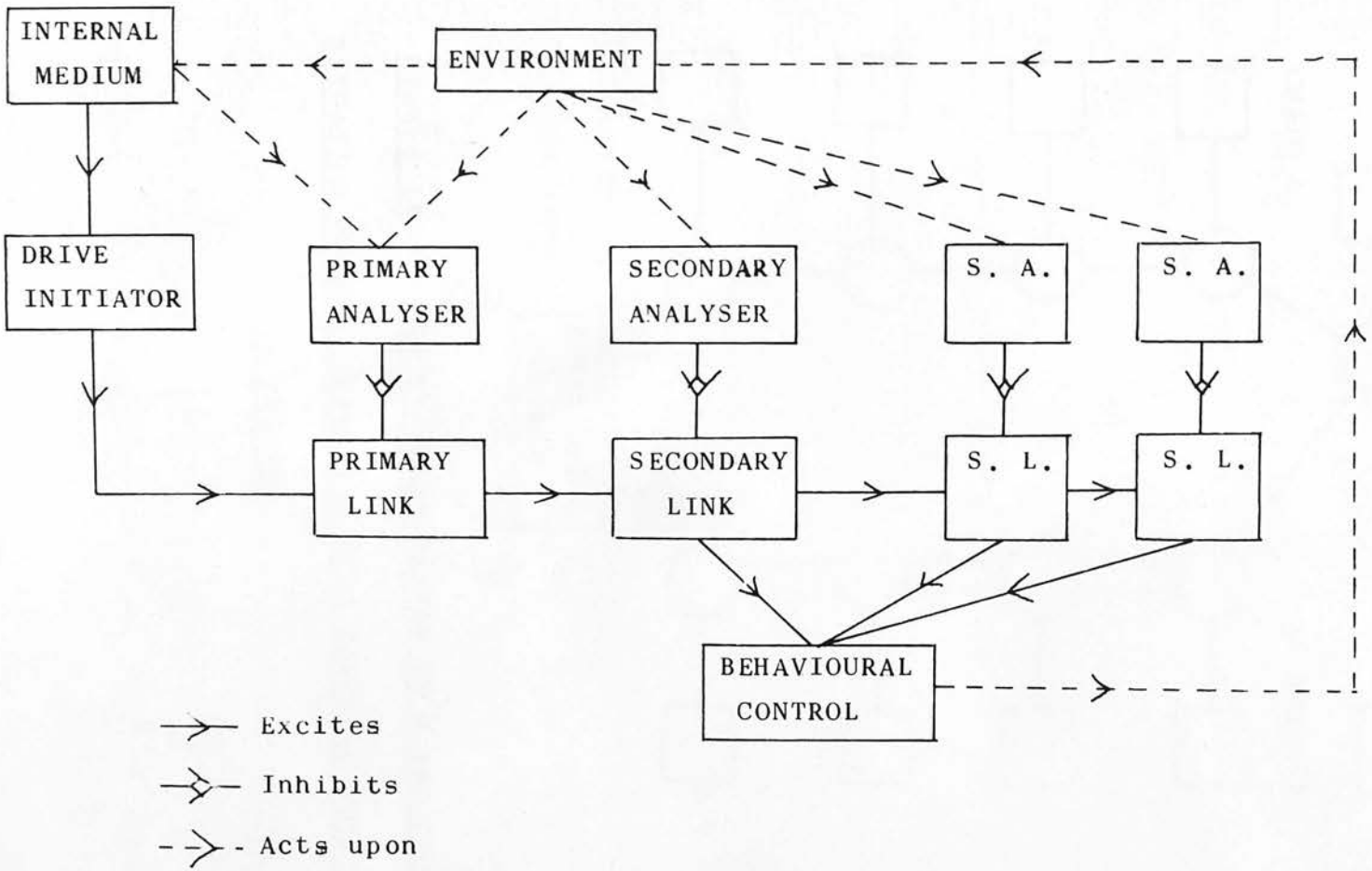
Now, according to Hull, reinforcement is provided by need reduction. Hunger drive will motivate the animal to eat, and the reduction of this need will reinforce the eating of either diet. However, R_v will in addition be reinforced by reduction of vitamin need, and over a period of time this will strengthen the sequence $S_v - R_v$ more than $S_d - R_d$. The situation is a little more complex than this in that the stimulus S consists of such factors as position, taste, colour, and texture. If the position of the diets is altered each day it implies that on Day 1 the response of eating on the left is being reinforced, on Day 2, eating on the right and so on, and at first sight it might seem that cognitive learning is also involved. However, irrelevant stimuli will sometimes be

reinforced, and sometimes not be reinforced, and ultimately only the significant stimuli, that is, those which are always associated with the need reduction, will elicit the response. This may explain why Lat (1967) found a significant relationship between discriminative learning and ability in self selection situations. The comparatively long delay between the response and need reduction, which is the case following the ingestion of most dietary components, need not necessarily imply ineffective reinforcement, in view of the findings of Garcia et al., (1967) and Rozin (1969).

Deutsch (1960) has constructed a cybernetic model of behaviour which is specifically intended to relate to inborn tendencies which are appetitive. The model is constructed according to the schema shown in Fig. 12. Changes in the internal medium lead to activity in the drive initiator, which in turn passes excitation to the primary link. This excitation travels on down the chain of links, provided they are not being stimulated by their associated analysers, which are responsive to specific cues in the environment. When a secondary link receives stimulation from its analyser, and simultaneously excitation from a primary link, it ceases to transmit excitation along the chain and instead switches it to behavioural control. Motor output is varied in such a way as to maximise stimulation from the analyser. Transmission of excitation continues until the primary link is switched off, which occurs when it is stimulated by its own analyser. This generally takes place during the performance of consummatory behaviour, such as the act of drinking by a thirsty animal. The connections between the links

FIGURE 12

SCHEMA OF DEUTSCH'S STRUCTURAL MODEL OF BEHAVIOUR



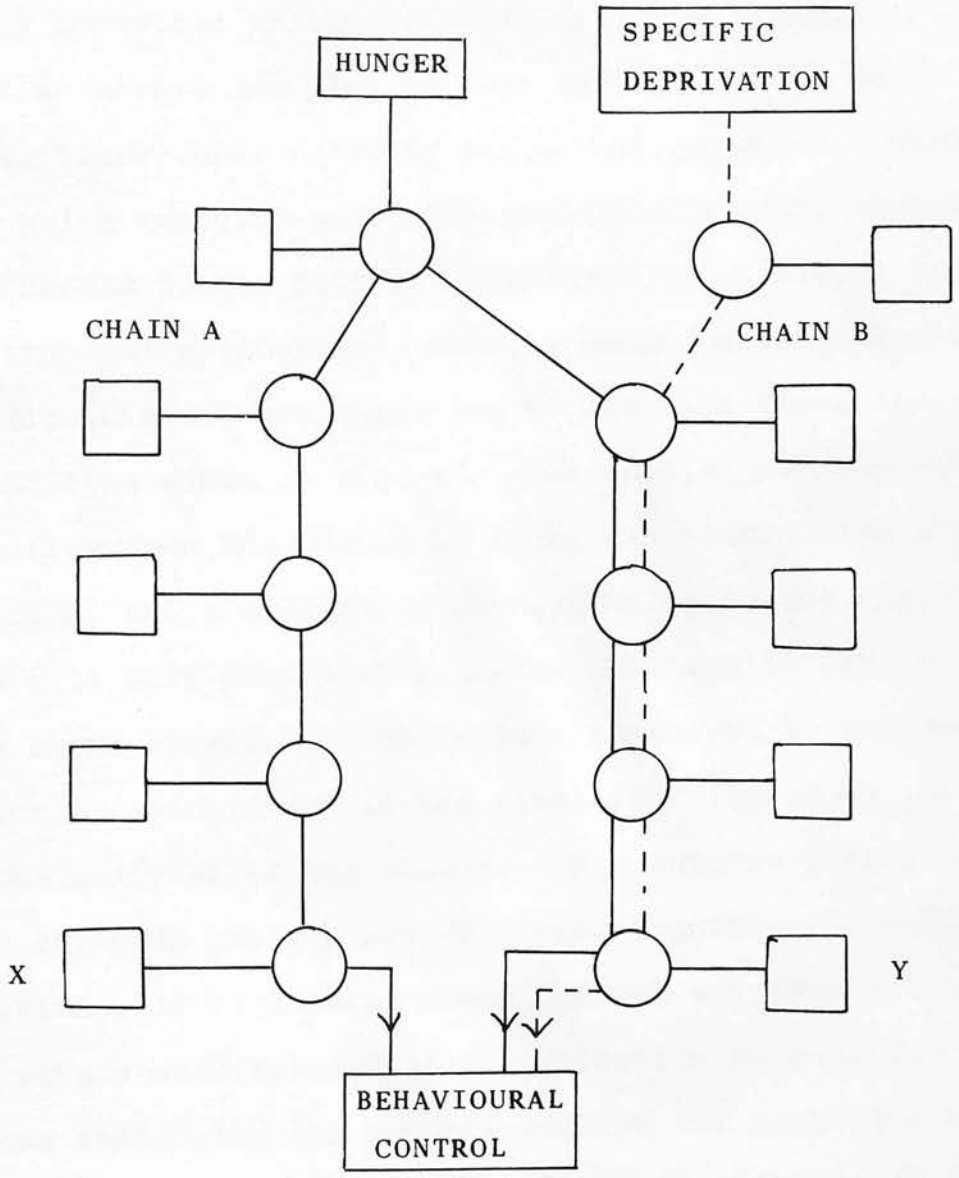


Figure 13. MODEL CORRESPONDING TO A TWO-CHOICE SITUATION WHERE DEPRIVATION OF A SPECIFIC NUTRIENT IS PRESENT.

may be either inherited or can be built up by learning. The model implies that an animal can pick up cues distant from a goal, and then performs behavioural activity which carries it to a nearer cue, the process being repeated until the goal is achieved, when the analyser connected to the primary link fires and activity ceases.

In a two choice situation, when a hunger drive and a drive for a specific nutrient are operating at the same time, the model will take the form shown in Fig. 13. Excitation corresponding to hunger will flow down the chains of links associated with both choices A and B, while excitation corresponding to the specific deprivation will only flow down B. The chains will have been built up as shown because a link becomes connected to another when the cessation of stimulation in the first is followed by the stimulation of the analyser of the second. Now suppose that cue x in chain A and cue y in chain B are presented together to the animal. More excitation will be flowing along chain B and thus the link associated with y will transmit more excitation to behavioural control, thus maximising its own stimulation and resulting in an approach to cue y. The end result will be consumption of the food containing the specific nutrient, with which the chain B of links is associated. The bias in favour of Chain B will obviously be stronger in cases where feeding is ad libitum, for then the excitation corresponding to specific deprivation will be relatively more influential than in cases where there is a very powerful hunger drive. It appears therefore that the structural model proposed by Deutsch can be readily modified to deal with the problem posed by the learning of a preference for food containing a specific nutrient,

provided that there is a primary link which can be switched off by an analyser sensitive to some property of the nutrient. This model of Deutsch's is able rather neatly therefore to sidestep the problem of reinforcement. If one does not accept Deutsch's model, in this particular case, on the grounds that there is insufficient evidence that deficiency of calcium leads to a specific inborn appetitive tendency, or 'drive', then one is left with the problem of the nature of the beneficial effect, or positive reinforcement, produced by the ingestion of calcium.

When growing chickens are deprived of calcium a number of changes occur. Over the six-week period of deprivation which was used in most of these experiments, the level of plasma calcium falls from about 12-14 mg/100 ml. to 8-10 mg/100 ml. If deprivation were extended, the fall would continue, to the point at which the animal shows hypocalcaemic tetany. This has frequently been reported in other species, and in fact was observed in one chicken, which showed a typical convulsion with torticollis, when it was disturbed by approach or by a sudden noise. As deprivation continues, there is a progressive decalcification of the bones of the body, beginning with the ribs, sternum and pelvis, and ending with the vertebrae, scapula and clavicle (Taylor, 1964). In addition, the parathyroid glands show considerable enlargement and engorgement, which is associated with the increased output of parathyroid hormone, and it is this that keeps the level of plasma calcium fairly high at the expense of the bones (Urist, 1959).

There would appear to be at least two effects of calcium ingestion by a deprived bird which are potentially positively rein-

forcing. It is probable that the lowered level of plasma calcium will have some central effect some time before overt hypocalcaemic tetany is seen. In humans acute anxiety produces a number of physiological and psychological symptoms, and these are identical to those seen in a state of pre-tetanic hypocalcaemia (Pitts and McClure, 1967). The infusion of sodium lactate can lead to an attack of anxiety in susceptible individuals, probably because the lactate in some way makes the calcium ion less available at the surface of excitable membranes of nerve cells.

If, therefore, calcium deprivation sets up a state of anxiety in the chicken, and the ingestion of calcium leads to anxiety reduction, it would be a powerful positive reinforcement. This hypothesis can also be viewed in the context of arousal levels. Berlyne (1967) reviews evidence suggesting that reinforcement may be equated with changes in arousal. It seems that a moderate increase in arousal is reinforcing, whereas a large increase is aversive. A fall in arousal can also be reinforcing if the basal level is very high, so it is suggested that there is an optimum level of arousal, and that any factor which leads to a change towards this level has reinforcing value. There seems good reason to suppose that hypocalcaemia leads to heightened arousal, since the calcium ion has a stabilising effect on the membrane potential of neurones (Frankenhaeuser and Hodgkin, 1957). It is therefore possible that the reinforcing effect of calcium in a deficient animal is mediated through a fall in high arousal levels, rather than by means of anxiety reduction. It should be borne in mind, however, that discussing mechanisms in terms of 'anxiety' or 'arousal' may merely be

two different ways of describing the same phenomenon.

Another beneficial effect of calcium ingestion could be linked with bone calcification. It is possible that extensive decalcification could lead to bone pain, and clearly a reduction in this pain would be positively reinforcing.

A third possibility is that the calcium ingestion does not have a specific effect at all. Calcium ions have a vital role in establishing and maintaining membrane potentials throughout the body, and it could be that the effect of increasing calcium concentrations is to produce a generalised sense of 'well-being'.

A number of experiments were performed in an effort to distinguish between these three hypothetical sources of reinforcement. No evidence was obtained to support the theory that bone pain was of any importance. Whereas mammals suffering from advanced rickets spend much of their time lying down, and when they can be persuaded to move do so slowly and stiffly, careful observation of the calcium-deprived chickens did not reveal any difference between them and normal controls. It was not possible to produce analgesia using recognised drugs like morphine and aspirin, and in any case fowls appear to be indifferent to shock and pain of a degree which would cause prostration in mammals. The alleviation of bone pain is also ruled out by the results from Experiment 11, for although the subcutaneous injection of a very small amount of calcium (50 mg) considerably delayed the onset of calcium preference, this amount would produce some 160 mg. of bone, and any effect on bone paid would be minimal, as this is only about 0·25% of the total skeleton weight of a normal bird.

Pilot experiments offering birds a choice between 10% ethyl alcohol and water suggested that calcium deprivation might be correlated with increasing alcohol consumption. Various workers have shown that alcohol consumption in a two choice situation increases under stress in an anxiety-producing situation in cats (Masserman and Yum, 1946) and in rhesus monkeys (Clark and Polish, 1960).

Learning by domestic rats in an avoidance situation, which is dependent upon anxiety reduction, is impaired by the ingestion of substantial doses of alcohol (Adamson and Black, 1959). However, calcium-deprived chickens which have only 10% ethyl alcohol to drink, show only a very slight impairment of their ability to select a calcium-supplemented diet (Experiment 15). Little is to be gained in using stronger alcohol concentrations since they are so aversive that the birds reduce their fluid intake, and thus ingest little, if any, more alcohol. Since alcohol has depressant, as well as anxiety-reducing effects, the experiment was repeated, using SU 9064, which is an effective tranquilliser in the fowl. There was no evidence that any impairment of calcium selection occurred, and one can only conclude that these findings do not support the theory that anxiety-reduction is an important source of positive reinforcement in the learning of a calcium preference.

Unsatisfactory though it may be, it appears probable that the third explanation is the most likely, namely that the effect operates through a generalised sense of 'well-being'.

Experiment 10 was performed in order to plot the time course of the reinforcement. Birds forced to eat supplemented diet for periods of 1 or 2 days did not select the supplemented diet more

effectively over the subsequent choice period than did birds which had been forced to eat the deficient diet. However, a four-day exposure to the supplemented diet did reinforce calcium-diet consumption significantly more than exposure to the deficient diet. At first sight this would suggest that need-reduction becomes effective in establishing a calcium preference with a duration of between 2 and 4 days. Too much weight should not, perhaps, be placed on this finding because there was a difference in methodology between the two parts of the experiment. In the case of the 2 day exposure the boxes were not exchanged from left to right, and in the case of the 4 day exposure they were. Thus with a 2 day exposure one may be reinforcing a positional preference as well as a dietary preference. In point of fact this is not borne out by the results, since 4 out of 5 birds showed a calcium preference on Day 2 of the choice period, when, if they had developed a positional preference, they should have exhibited a deficient-diet preference. The other objection to this experiment is that it will not yield useful information if deprived birds develop a novel-diet preference, as suggested by Rodgers (1967), for, as the birds exposed to the calcium diet develop a calcium preference, the birds exposed to the deficient diet develop a learnt aversion.

Unlike rats, though, deficient chickens do not appear to exhibit a preference for an arbitrary novel diet. On occasion a bird may continue ingesting a calcium-deficient diet over a 10-day choice period in preference to a novel calcium-supplemented diet. In addition, chickens select the calcium-supplemented diet with equal facility even though both of the choice diets are novel (Experiment 12),

and also when the supplemented diet is familiar and the deficient diet is novel (Experiment 13). In none of these experiments is there any suggestion that the novel diet has any inherent attraction, and this is true of sodium and thiamine deficiency too. It is true that the thiamine-deficient birds in Experiment 25 exhibit a much higher intake of the supplemented diet on Day 1 than do the controls, but it is still well below 50%, and this could be accounted for by the fact that thiamine produces a beneficial effect of very rapid onset in deficient animals.

With these findings in mind, one has to conclude that what might be called the 'strategy of selection' in rats, does not operate in chickens. Rozin and his co-workers are not altogether consistent about this. In their early papers, neophilia is seen as a tendency which is displayed by deficient animals, and which is primarily adaptive since it increases the chances that they will ingest a source of the essential component that is lacking. The positive reinforcement produced by this ingestion then increases the intake of the novel diet. If the novel diet is also deficient then the tendency to ingest it gradually wanes. In later papers (Rozin 1967a, 1968), he reports that both poisoning and deficiency actually produce an avoidance of novel diets, or neophobia, and that any neophilia seen is in fact only relative, being related to an even more intense learnt aversion to the harmful diet. The situation is made even more complex by the fact that thiamine-deficient rats do not show a preference for a solution of thiamine as opposed to water, even when need reduction has occurred and the deficiency has been corrected. This later work on thiamine-deficiency in rats

does mean that the situation is now back in the melting pot again. However, these findings may be applicable only in the rat, since similar experiments have not yet been reported in other mammals, and there is no suggestion that these phenomena occur in the chicken.

To summarise, calcium selection by the deficient chicken can most readily be explained in terms of a learnt preference, but the role of reinforcement in the development of this preference remains unclear. The most promising approach is perhaps to view it in the context of a reduction of a very high arousal level, as proposed by Berlyne (1967) and others. Some additional evidence is provided for this by Urist (1959) who reported that adrenal glands of calcium-deprived hens were greatly increased in size over those of normal birds (in some cases the increase exceeded 100%), and also abnormally vascular, suggesting hyperactivity. It seems probable that the release of these large amounts of corticosteroids would activate and maintain a state of chronic arousal, and that when calcium ingestion occurs, this state of chronic stress is reversed, and there is a non-specific beneficial effect, which results in a gradually lowered level of arousal.

In view of the fact that thiamine-deficient rats fail to select solutions of thiamine, it is interesting that calcium-deprived chickens also fail to choose calcium-solutions rather than water (Experiment 17). Since, however, this is not an absolute difference between diets and fluids, as they do show a preference for calcium carbonate suspensions in water, it is easiest to explain in terms of Young's hedonic principles. This is given added weight by the finding (Kare and Pick, 1960) that fowls were very much more sensitive

to offensive flavours in water than in food, since it appears that chickens find the taste of calcium acetate and calcium lactate solutions exceedingly unpleasant, and if a solution is highly aversive, too little is consumed to produce an appreciable beneficial effect. Even when the calcium solution is available alone for a period of 4 days, so that the bird has no option but to consume it and to experience the beneficial effect, and then after that, it is paired with 0·05% quinine hydrochloride, which normal birds find less aversive than calcium borogluconate, the deprived birds still show no preference for the calcium solution. These results certainly suggest that when two fluids as aversive as these are offered, the birds appear to select whichever one they find the more palatable.

Only a brief experiment was carried out on the influence that calcium deprivation might have on general activity, which was measured in a stabilimeter cage (Experiment 9). The results obtained were equivocal, possibly because of environmental disturbances, though there was a trend towards higher activity under conditions of increased deprivation. In any case a stabilimeter, though the only feasible method when dealing with chickens, may not be the most sensitive device for detecting increased activity. Weasner, Finger and Reid (1960) have shown that activity changes can, at least partly, be a function of the recording device, and that an activity wheel may record an increase ten times as great as that seen in a stationary cage. Campbell et al. (1966) have, however, observed a rise in the activity of chicks with both food and also water deprivation, when activity was recorded in a stabilimeter, so it seems that this method

can, in the appropriate conditions, yield useful information.

The influence of specific deprivation upon a more directed form of activity was readily demonstrable. Calcium deprivation led to an increase in pecking responses in an open arena, but did not markedly alter the pattern of pecking from that observed in the controls (Experiment 8). It seemed that the effect of the deprivation was two-fold - it lowered the threshold of the response, so that stimuli which were ignored by normal chickens elicited pecking in the deprived birds, and also reduced the effect of satiation factors, so that the birds continued pecking for a longer period. Activity in the general sense was not necessarily increased in this situation - the deprived birds certainly entered fewer squares of the arena, and may even have expended less energy than the controls, which showed much more escape behaviour. It is interesting that a similar increase in pecking tendency is seen in sodium-deprived chickens (Experiment 23), indicating that this response is not a specific behaviour pattern triggered by calcium deficiency, as is suggested by Lorenz (1966), since calcium and sodium deficiency both elicit what, to a subjective observer, appear to be identical patterns of behaviour. There do seem to be some unlearned components, however, because calcium-deprived, sodium-deprived and control birds all exhibit basically similar preferences in their stimulus choice pattern, when presented with these novel stimuli.

The influence of parathormone on directed pecking was examined in Experiment 8.4, in an attempt to determine whether the increased pecking tendency observed in calcium-deprived birds was causally related to the high levels of circulating parathyroid hormone, or whether

it was primarily dependent upon the low level of plasma calcium. The injection of parathormone did not increase pecking activity in normal birds, indicating that the altered behaviour seen in calcium-deprived birds is not produced by the effect of elevated parathyroid hormone levels acting directly. Parathormone had no effect in deprived birds either, pecking activity remaining at a high level following the injection. However, although parathormone raises plasma calcium levels substantially at the dosage used, in normal birds (Hertelendy, 1962), it does not have any effect in deprived birds, probably because endogenous parathyroid hormone levels are already so high that calcium is being mobilised from the bones to the greatest possible extent. Thus the hypothesis that increased pecking activity is produced by the central effect of lowered plasma calcium levels must remain unproven.

Even if this is the mechanism underlying the increased activity, it is clearly not a specific effect, as a similar increase is observed in sodium deficiency, implying that both calcium and sodium deficiency do, in this respect, have a final common path.

In other respects, however, calcium and sodium deficiency in the chicken are quite different. Sodium-deprived chickens exhibit no preference for sodium-supplemented diets, nor do they select sodium chloride solutions in preference to water. The tests were all carried out using sodium chloride, which is the commonest natural source of sodium, and also so that the experiments were comparable with those carried out in the rat. It seemed unlikely that other sodium salts would be preferred if sodium chloride was not selected.

Workers such as Engelmann, 1934; Duncan, 1962; Kare and

Ficken, 1963; report that the normal fowl either prefers or is indifferent to concentrations of salt up to 0·9%. This work could not be confirmed and it was found that, under the conditions of Experiment 22, normal chickens exhibited an aversion to a 0·7% sodium chloride solution. A period of sodium deprivation lasting 42 days (Experiment 21) did not alter this aversion to any material degree. Experiment 21 shows that this aversion is cue-dependent to a remarkable extent, the cue being a blue dye, present initially in the sodium chloride solution, which was switched to the water after 7 days. The graph in Fig. 10 shows how the sodium chloride intake of the deprived birds rises from about 3·5% on Day 7 to 85% on Day 8, following which it falls back to around 25% after a further 3 or 4 days. At first sight this pattern suggests that the chicken is associating some post-ingestional aversion feedback with the stimulus pattern of the solution - the blue colour being its most prominent property. It seems unlikely, however, that such a weak solution could have an aversive effect over such a short time, particularly in the deprived birds, and a more plausible explanation is that the birds have difficulty in distinguishing between the salt solution and water, and that what they have learnt is the ability to distinguish between the two fluids, rather than an aversion for the salt. The aversion was present the whole time.

The existence of this aversion is, in itself, a problem in the context of the rat findings. Rats show a typical preference-aversion curve with increasing salt concentration, whether the testing is single comparison (Weiner and Stellar, 1951) or simultaneous choice (Bare, 1949). The maximal preference is at a

concentration of around 0·8%, and above about 1·1% an aversion is exhibited. This preference is not adaptive, indeed Nelson (1947) gave young rats continuous access to 0·8% NaCl and to water, and found that after 13 weeks they weighed less and had damaged kidneys, compared to controls with access only to water. Deutsch and Jones (1960) have proposed that addition of salt 'dilutes' the water as far as the receptor mechanisms are concerned, so that more saline has to be drunk in order to produce the same 'water' signal. More recent work (Falk and Titlebaum, 1963; Chiang and Wilson, 1963) has failed to support this hypothesis, and suggests that a cognitive element may be implicated.

Experiment 22 indicates that chickens show an aversion to salt at a concentration similar to that at which rats exhibit their maximal preference. This could be so because of differences in the receptor, conductor or perceptual systems, but it cannot be explained in terms of a fundamental difference between birds and mammals, in view of Duncan's (1962) description of a preference-aversion curve in the pigeon, which bears a close relationship to that seen in the rat, and also the finding that dogs prefer water to saline, and that cats can be divided into two groups, half preferring saline to water and the other half water to saline (Towbin, 1967). Indeed, Towbin's description of the behaviour of dogs mirrors exactly that described in the chicken (Experiment 22). When dogs were given a choice, they exhibited a preference for water. However, when offered only saline or only water to drink when thirsty, they consumed more than twice as much saline as water.

It may indeed be possible to integrate these different strands

into a comprehensive theory which can be applied to a variety of different species. The 'diluted-water' hypothesis is simple and elegant, and is supported by neurophysiological evidence in the rat (Zotterman, 1956). This could be the primary factor controlling fluid intake in a situation where there is no choice, that is, when either water alone or saline alone is presented. Rat, chicken and dog, at least, all consume more saline than water in a no choice situation, and it may be that palatability factors are of lesser importance under these conditions. This is true of the chicken both under ad lib. conditions and also when being tested using the single comparison method, with water and salt solution available for a limited period on alternate days. When the animal is able to exercise a choice, however, it appears that palatability assumes a more important role, and inter-specific differences appear according to whether the individual animal finds a saline solution palatable or not. From evidence cited by Towbin (1967) in the case of the cat, it seems that there may also be intra-specific differences in the acceptability of saline, and in the case of chickens too some birds appear to find the salt solution more aversive than others. This sort of theory must remain very speculative at present, because of the paucity of behavioural studies dealing with comparative preferences in a variety of different experimental situations.

In view of the findings of Experiments 19, 20 and 21, in which birds had failed to select either diets or solutions supplemented with sodium chloride, even though they had been deprived of sodium for periods of 5 or 6 weeks, a different method of inducing a state

of sodium deficiency was employed. Dietary deprivation results in only a very slight lowering of plasma sodium levels, whereas the technique described by Wolf and Steinbaum (1965), in which a weak solution of formalin is injected subcutaneously, leads to an appreciable loss of sodium from the circulation. The mean fall in the plasma sodium level of the chicken was about 12%, compared with a fall of around 6% in the rat, indicating that this technique is effective in the bird. Rats made sodium-deficient by this method show an avidity for salt in both food and drink, whereas functional sodium deficiency had no detectable effect in the chicken (Experiment 24). As suggested earlier, an explanation for these differences may need to be sought at the physiological level.

This behaviour exhibited by the chicken can only be described as a paradoxical response. These sodium-deficient birds in an open arena exhibit exploratory pecking which is superficially indistinguishable from that seen in calcium-deprived birds, and this response is particularly obvious in young sodium-deprived chicks who will cluster around a hand or other object placed in the brooder and engage in vigorous and sustained pecking activity, rather than showing escape and fleeing behaviour like normal chicks. This exploratory activity gives every indication of being the first phase of a homeostatic response, the searching phase, which will be followed by identification of the appropriate nutrient and finally its ingestion. However, in the case of sodium the first phase only appears to be present, - identification and ingestion are inexplicably absent. Palatability cannot be an important factor in selection of a diet, since the birds were indifferent to the

presence of salt during the control period.

The experiment investigating selection of a thiamine-supplemented diet by thiamine-deficient birds was rather brief in comparison with those on calcium and sodium preference, but the findings were relatively clear-cut. As in the case of the rat, administration of oxythiamine was an effective means of inducing a deficiency, at least as judged by a decline in food intake, though a much larger dose over a much longer period was necessary.

Group selection of thiamine by these birds followed very much the same pattern seen in the case of calcium, that is, intake was low on Day 1 but rose rapidly to about 85% by Day 3. Indeed, the only notable difference lay in the fact that the switch to thiamine-supplemented diet was, if anything, even faster. It may be that this can be explained on the grounds that the effects of thiamine in a deficient animal follow a very short time course. Thiamine can exert an effect on such physiological parameters as heart rate in a deficient animal in as short a time as 30 minutes (Rozin, 1967).

Although the record of food intake for the group of deficient birds appears to follow the form of a learning curve over the first three days of selection, it differs from the findings in the case of calcium in that there is a substantial difference between the deficient group and the controls even on Day 1, and it could be argued that these results could be interpreted as demonstrating some degree of novelty preference. In Experiment 25.2 the proportion of thiamine diet ingested expressed as a percentage is 26% in the deficient group compared with 3% in the controls, while in Experiment 25.3 it is 38% and 13% respectively. In both cases the deficient

group are ingesting less than half of their intake in the form of the thiamine diet, but it could nevertheless be argued that they are still showing a relative (though not an absolute) preference for novelty, and that in this respect the results resemble those obtained in the rat. At first sight, therefore, this experiment does not rule out the possibility that novelty preference may play some part in the selection of thiamine by the chicken, even though a learning interpretation appears to be compatible with most of the findings. With this in mind, when a choice was first presented to the chickens in Experiment 25.3, careful observation was carried out to determine the initial preference of each bird. Of the 9 deficient birds, eight began to eat first from the diet which lacked thiamine, indicating conclusively that initial preference for a novel diet does not play an appreciable part in the selection of thiamine. The fact that there is a considerable difference between the two groups on Day 1, unlike the findings in the selection of calcium, must be a reflection of the more rapid development of the preference for thiamine.

Perhaps this discussion should be concluded by considering these findings in an evolutionary and environmental context. Evidence regarding the diet and nutritional requirements of the jungle fowl, from which the domestic chicken has been derived, is scanty, but in general its food is of a varied nature (Collias, 1967) and with the exception of calcium most of its obvious requirements should be readily met without special selection. Calcium turnover and requirement in laying hens is very considerable (1 gm.

per egg in the case of jungle fowl, Haddon, 1945; Gilbert, 1970) and in view of the fact that Collias has reported finding fragments of snail shell in the crop it seems highly probable that a special selection is exercised in this case. It seems doubtful that a similar situation applies in the case of sodium. A jungle fowl will probably lay between 20 and 30 eggs in a breeding season (Haddon, 1945), each weighing about 30 gm. and containing 25 mg. of sodium (Leonard, 1969). The total loss of sodium will therefore amount to less than 750 mg. during this period, and should be negligible during the remainder of the year, owing to renal efficiency in sodium retention. Thus the fowl, in its natural environment, would never be in a state of sodium need. The same is probably true of thiamine, as seeds of various kinds form a considerable proportion of its diet.

One may conclude that a specific appetite, in the fowl, should be viewed not as a basic physiological drive, but as a manifestation of a rather general ability, an adaptive response to deficiency which can be activated in the appropriate circumstances, but which appears to have certain inherent limitations. These may be related to nutritional requirements in an evolutionary context.

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APPENDIX

This appendix contains details of the composition of the diets which were used in the course of the deprivation experiments, and also the complete details of the results obtained in certain of the experiments, because the tables of results were too bulky to be included in the body of the text. There is a cross-correspondence of numbering, that is, Table 2.1 in the text refers to Table 2.1A in the appendix, and so on.

COMPOSITION OF DIETS

Composition of Diet 1 (low calcium)

Ingredient	%
Wheat meal	35·00
Maize meal	50·00
Lactic C casein	13·00
Sodium chloride	0·50
Disodium hydrogen phosphate	1·00
Zoamix	0·05

Vitamins (per 100 Kg)	
Rovimix A + D_3	3·2 gm
Rovimix A_{50}	0·8 gm
Rovimix E_{25}	10 gm
Menaphthone	130 mg
Riboflavin	400 mg
Nicotinic acid	2·8 gm
Pantothenic acid	1·0 gm
Cyanocobalamin	3 mg

Minerals (per 100 Kg)	
Copper sulphate (hydrated)	1·4 gm
Potassium iodate	67 mg
Ferrous sulphate (hydrated)	22·3 gm
Magnesium carbonate	95 gm
Manganese carbonate	20·9 gm
Zinc oxide	6·2 gm

Composition of Diet 2 (low calcium)

Ingredient	%
Maize meal	72·65
Soyabean meal	25·00
L-lysine monohydrochloride	0·20
DL-methionine	0·15
Sodium chloride	0·25
Disodium hydrogen phosphate	1·75

Vitamin supplement, mineral supplement and zoamix as Diet 1.

Composition of Diet 3 (low calcium)

Wheat meal	76·00
Soyabean meal	22·00
Sodium chloride	0·25
Disodium hydrogen phosphate	1·75

Vitamin supplement, mineral supplement and zoamix as Diet 1.

Composition of Diet 4 (low sodium)

Wheat meal	24·56
Barley meal	24·79
Maize meal	21·84
Soyabean meal	24·91
Calcium hydrogen phosphate	2·35
Calcium carbonate	0·96

Vitamin supplement, mineral supplement and zoamix as Diet 1.

Composition of Diet 5 (Breeders ration)

Ingredient	%
Maize meal	25·00
Wheat meal	10·00
Oatmeal	25·00
Middlings	10·00
Bran	10·00
Fish meal	6·25
Dried yeast	2·50
Dried skim milk	3·75
Dried grass	2·5
Limestone	2·5
Cod Liver Oil	2·0
Mineral Mix	2·5

Mineral Mix	%
Ferrous sulphate	2·46
Manganese sulphate	4·82
Limestone flour	62·59
Bone flour	13·77
Sodium chloride	14·74
Potassium iodide	0·065
Cobalt sulphate	0·065
Copper sulphate	1·49

Composition of Diet 6 (low thiamine)

Ingredient	%
Casein	25·00
Gelatin	15·00
Starch	30·00
Sucrose	10·00
Corn Oil	9·00
Sodium chloride	0·50
Potassium chloride	0·50
Calcium hydrogen phosphate	3·75
Calcium carbonate	0·55
DL-methionine	0·55

Vitamin Mix (gms/100 kg)	
Rovimix A + D_3	17·60
Menaphthone	0·41
Tocopherol acetate	7·93
Riboflavin	0·90
Ca pantothenate	3·00
Choline chloride	501·00
Nicotinic acid	12·00
Folic acid	0·22
Pyridoxine HCl	0·90

(Contd.)

Diet 6 (contd.)

Ingredient (contd.)	%
Mineral Mix	0·23
Vitamin Mix	0·54
Water to 100·00	

Mineral Solution (gms/100 kg)	
Sodium fluoride	0·442
Potassium iodate	0·084
Sodium molybdate	0·250
Sodium selenate	0·022

Mineral Mix	
Copper carbonate (basic)	1·85
Zinc Oxide	10·00
Manganese carbonate	24·50
Ferrous sulphate	29·70
Magnesium carbonate	163·30

Vitamin solution (gms/100 kg)	
Biotin	0·035
Cyanocobalamin	0·004

COMPLETE DETAILS OF EXPERIMENTAL RESULTS WHERE THESE HAVE NOT BEEN INCLUDED IN THE TEXT

Table 2.1A

Results of Experiment 1.

Selection of 2% calcium carbonate

The amount eaten is given in gms. In each case the calcium-supplemented diet is given first, and the deficient diet second.

Experimental (Calcium deprived) birds

No. of Bird		Day 1	2	3	4	5	6	7	8	9	10	No. Ca	% Ca
B4345		72	72	69	70	77	85	25	80	84	80	8	69·9
	L+	49	21	49	16	25	19·	76	7	27	18		
B4340	+	81	62	85	48	95	89	100	61	113	94	7	59·9
	L	62	52	73	58	36	65	42	63	33	69		
B4339		27	84	79	90	112	116	115	110	117	139	9	83·8
	L+	112	28	47	0	0	1	2	0	0	1		
B4350	+	61	35	90	11	81	29	93	30	88	68	5	62·3
	L	22	72	22	72	6	69	0	58	1	32		
B4336	+	20	70	70	62	90	90	101	102	96	123	8½	66·8
	R	118	39	70	32	23	46	19	20	26	16		
B4337	+	139	122	123	82	64	97	107	77	89	81	9	76·8
	R	14	21	23	46	38	44	8	25	19	59		
B4349		5	89	15	75	6	91	14	86	38	100	5	49·1
	R+	129	6	100	7	100	3	98	6	83	6		
B4344		82	48	122	66	67	107	111	98	117	138	8	78·5
	R+	72	52	15	53	45	9	16	0	0	0		

Totals

Ca.diet	487	582	653	504	592	704	666	644	742	823
D. diet	578	291	399	284	273	256	261	179	189	201
Ca diet as a%	45·7	66·7	62·1	64·0	68·4	73·3	71·8	78·3	79·6	80·4

NOTE: R = calcium diet initially on right
L = calcium diet initially on left
\+ = box first sampled

Table 2.1A (Contd.)

Normal Control birds

No. of Bird		Day 1	2	3	4	5	6	7	8	9	10	No. Ca	% Ca
B4343		89	123	105	85	44	30	26	42	-	-	(4)	57·4
	L+	87	24	46	17	71	54	68	36	-	-		
B4338		25	32	16	63	70	58	58	70	92	98	6½	41·2
	L+	130	85	111	35	27	47	58	19	10	10		
B4342	+	128	77	113	74	84	99	104	16	31	0	6	74·7
	L	19	7	0	0	0	0	1	83	52	84		
B4348	+	135	59	145	105	145	134	143	134	124	161	8	78·3
	R	99	72	49	23	20	19	1	13	35	25		
B4347		7	5	0	4	5	5	0	0	0	0	0	2·3
	R+	141	106	126	83	100	102	112	85	121	116		

Totals

Ca diet	384	296	379	331	348	326	331	262	247	259
D diet	476	294	332	158	218	222	240	236	218	235
Ca diet as a %	44·7	50·2	53·3	67·7	61·5	59·5	58·0	53·1	53·1	52·4

BATCH 2.

Experimental birds

No. of Bird·		Day 1	2	3	4	5	6	7	8	9	10	No. Ca	% Ca
B4968		3	74	1	89	0	73	81	83	95	75	7	76·4
	L	51	0	72	0	53	0	0	0	1	0		
B4707		7	66	47	100	86	88	106	95	93	84	9	92·0
	L	33	2	32	0	0	0	0	0	0	0		
B4695		17	52	58	87	60	79	63	74	63	48	9	7·19
	L	49	12	30	17	25	1	30	14	16	41		
B4702		2	3	5	0	0	1	1	0	0	0	0	1·5
	L	71	78	81	112	74	52	80	86	71	66		
B4699		4	15	55	96	56	77	50	57	31	12	5	47·0
	L	81	81	54	18	41	4	53	26	84	68		

[Contd.

NOTE: R = calcium diet initially on right
L = calcium diet initially on left
+ = box first sampled.

Table 2.1A (Contd.)

Batch 2 (contd.)
Experimental birds (contd.)

No. of Bird		Day 1	2	3	4	5	6	7	8	9	10	No. Ca	% Ca
B4579		59	91	97	99	90	95	103	105	99	73	9	98·0
	L	17	0	1	0	0	0	0	0	0	0		
B4585		80	93	97	147	135	113	126	119	115	97	9	99·1
	R	5	5	0	0	0	0	0	0	0	0		
B4704		38	50	83	91	87	70	88	91	89	75	9	88·7
	R	35	22	6	10	0	10	0	0	4	0		
B4581		36	80	110	136	125	114	129	135	126	112	9	92·1
	R	51	30	9	0	0	0	1	0	2	1		
B4583		83	87	93	104	80	70	78	62	76	64	9	86·3
	R	7	11	14	6	15	4	16	12	33	8		
B4696		27	64	47	87	70	84	64	92	98	94	8	76·4
	R	67	22	63	21	20	3	29	0	0	0		
Totals													
Ca diet		356	675	693	1036	789	864	889	913	885	734		
D. diet		467	263	362	184	228	74	209	138	211	184		
Ca %		43·3	73·0	65·7	84·8	77·6	92·1	81·1	86·9	80·8	80·0		
Normal Controls													
B4967		40	18	6	7	2	0	0	0	0	0	0	6·1
	L	102	125	128	127	114	95	109	115	111	95		
B4580		105	49	41	43	74	54	29	69	25	29	3	40·5
	L	56	70	104	97	53	52	100	56	97	73		
B4584		124	11	86	118	100	87	112	123	127	106	8	97·0
	L	5	23	1	0	1	0	0	0	0	0		
B4577		66	52	40	73	29	66	64	65	46	67	4	48·7
	L	70	58	92	56	40	32	62	71	76	40		
B4700		50	40	48	116	127	115	104	102	92	37	6	66·4
	L	74	71	102	10	2	30	13	41	17	60		
B4701		106	31	114	109	116	115	111	135	106	58	7	86·1
	R	10	91	0	0	0	0	0	0	0	60		
B4709		37	92	30	68	35	68	36	88	24	59	5	42·9
	R	65	40	115	44	123	51	93	48	102	34		

[Contd.

NOTE: R = calcium diet on right
L = calcium diet on left

Table 2.1A (Contd.)

Batch 2 (contd.)
Normal controls (contd.)

No. of Bird	Day 1	2	3	4	5	6	7	8	9	10	No. Ca	% Ca
B4708	30	95	23	36	12	34	26	29	48	29	1	29·0
R	90	39	102	113	100	66	105	98	87	87		
B4705	5	83	46	92	20	42	38	40	100	76	4	48·3
R	90	1	85	35	86	76	77	73	32	26		
B4582	14	5	24	70	39	47	0	71	0	60	4	39·1
R	47	43	65	38	63	20	90	27	79	42		
Totals												
Ca diet	577	476	458	732	544	628	520	722	568	521		
D. diet	610	561	794	520	582	422	649	529	601	517		
Ca as a %	48·6	46·0	36·6	58·4	48·3	59·8	44·5	57·7	48·7	50·2		

Combined Totals of Batches 1 and 2

Experimental Birds

Ca diet	843	1257	1346	1540	1381	1568	1555	1557	1627	1557
D. diet	1045	544	761	468	501	330	470	317	400	385
Ca as a %	44·7	69·8	63·9	76·7	73·4	82·6	76·8	83·1	80·3	80·2

Normal Controls

Ca diet	961	772	837	1063	892	954	851	784	815	780
D. diet	1086	855	1126	678	800	644	889	765	819	752
Ca as a %	46·9	47·5	42·6	61·1	52·7	59·7	48·9	50·6	49·9	50·9

Table 2.2A

Blood Calcium Estimations

No. of Bird	Group	Ca level before Choice	Ca level after Choice
B4579	E	10·0	Not estimated
B4704	E	10·2	12·9
B4585	E	7·0	12·3
B4580	C	16·5	14·0
B4700	C	18·4	11·7
B4705	C	16·4	13·3

NOTE:
R = calcium diet on right

Table 3A

Batch 1.

Experimental Birds

No. of Bird	Side	Jly 4	5	6	7	8	9	10	11	12	13	Total	Ca %	Ca No.
B7045	L	53	47	112	128	132	138	116	119	135	137	1117	85·0	8
		96	84	16	0	0	1	0	0	0	0	197		
B7054	L	16	71	27	101	64	113	63	129	124	129	837	60·6	7
		65	52	66	32	61	17	71	93	55	32	544		
B7047	L	9	64	68	114	106	111	105	117	134	110	938	82·4	9
		101	49	41	7	0	2	0	1	0	0	201		
B7044	R	6	100	39	116	17	100	32	90	85	111	696	58·6	6
		97	12	77	7	101	21	83	39	41	13	491		
B7043	R	74	18	2	2	1	44	33	75	64	67	380	42·1	3
		37	47	72	86	79	67	52	38	30	15	523		
B7050	R	77	37	83	89	86	89	87	80	88	89	805	75·2	8
		31	64	24	16	17	27	19	26	26	16	266		
Ca diet Tot.		235	337	331	550	406	595	436	610	630	643			
D. diet Tot.		427	308	316	148	258	135	225	197	152	76			
Ca diet %		35·5	52·2	51·2	78·8	61·2	81·5	66·0	75·6	80·6	89·4			

Normal Controls

B7049	L	75	56	66	56	70	78	64	60	44	42	611	37·4	0
		84	101	110	137	108	103	91	87	91	109	1021		
B7046	L	59	57	67	26	82	21	73	40	34	86	545	35·4	2
		85	116	100	133	36	144	92	101	131	57	995		

[Contd.

Table 3A (Contd.)

Batch 1 (contd.)
Normal controls (contd.)

No. of Bird	Side	Jly 4	5	6	7	8	9	10	11	12	13	Total	Ca %	Ca No.
B7052	L	67	2	68	16	10	2	9	17	48	65	304	22·0	0
		86	147	72	117	133	132	120	126	80	66	1079		
B7051	R	30	27	0	65	0	42	1	43	1	123	332	22·8	1
		96	102	135	93	138	122	142	128	143	27	1126		
B7048	R	45	14	4	2	5	9	1	6	0	17	103	9·6	0
		83	87	94	98	98	115	103	105	103	85	971		
Ca diet Tot.		276	156	205	165	167	152	148	166	127	333			
D. diet Tot.		434	553	511	578	513	616	548	547	548	344			
Ca diet %		38·9	22·0	28·6	22·2	24·5	19·8	21·3	23·3	18·8	49·2			

BATCH 2
Experimental Birds

		Jly 14	15	16	17	18	19	20	21	22	23			
B7256	L	112	102	132	136	126	128	138	139	132	136	1281	99·1	9
		2	1	1	0	0	0	0	0	0	0	4		
B7258	L	20	59	19	85	29	90	39	95	54	87	577	78·1	7
		43	12	39	0	30	2	28	0	3	0	157		
B7259	R	0	84	25	77	46	82	67	102	88	112	683	65·4	8
		90	26	82	38	44	16	46	5	5	2	354		
B7260	R	72	76	80	86	79	80	84	100	87	96	840	94·3	9
		0	5	1	0	0	0	0	0	0	0	6		
B7261	L	79	65	87	92	91	100	110	112	104	108	948	92·7	9
		10	33	4	13	0	6	8	0	0	0	74		

[Contd.

Table 3A (Contd.)

Batch 2 (contd.)
Experimental birds (contd.)

No. of Bird	Side	Jly 14	15	16	17	18	19	20	21	22	23	Total	Ca %	Ca No.
B7263	R	29	77	79	90	79	94	89	107	77	104	825	90·0	9
		41	17	9	4	3	0	2	1	10	4	91		
B7264	R	70	48	92	111	80	94	91	118	88	112	904	94·0	9
		20	34	3	0	0	0	0	0	2	0	59		
B7265	R	40	60	70	78	88	65	87	67	63	82	700	71·7	9
		20	18	27	18	1	54	35	41	26	37	277		
B7267	R	32	71	61	100	86	113	89	117	84	114	867	83·7	9
		45	26	29	16	9	3	23	9	2	7	169		
B7282	L	8	99	81	101	76	99	96	111	99	105	875	75·7	9
		65	35	56	17	51	11	32	5	6	3	281		
B7284	R	63	82	105	121	114	111	121	120	99	116	1052	93·8	9
		31	17	2	0	0	0	12	6	0	2	70		
B7311	L	34	56	57	66	35	70	85	101	83	94	681	77·0	8
		31	27	38	23	43	17	8	6	7	4	204		
Ca diet Tot.		559	879	888	1143	929	1126	1096	1289	1057	1266			
D. diet Tot.		398	251	291	129	181	109	194	73	61	59			
Ca diet %		58·4	77·7	75·4	89·8	83·7	91·2	85·0	94·6	94·5	95·5			

Normal Controls

B7273	R	19	49	59	89	95	108	92	104	45	87	747	52·4	6
		67	60	75	79	52	67	80	75	50	74	679		
B7274	R	53	56	57	76	52	72	75	82	15	61	599	40·8	0
		51	80	110	115	96	92	92	102	33	95	866		
B7276	R	46	73	65	92	63	62	50	86	35	66	638	46·1	3
		70	72	83	84	84	88	87	60	49	71	748		
B7278	R	15	26	27	24	27	15	35	24	13	21	227	19·8	0
		76	86	93	107	75	117	89	115	58	105	921		

[Contd.

Table 3A (Contd.)

Batch 2 (contd.)

Normal controls (contd.)

No. of Bird	Side	Jly 14	15	16	17	18	19	20	21	22	23	Total	Ca %	Ca No.
B7283	R	56	35	17	49	23	31	20	62	18·	34	345	31·5	1
		53	70	84	86	91	90	91	59	56	72	752		
B7285	R	48	64	12	35	50	75	38	61	6	43	432	49·5	5
		36	21	85	46	47	24	60	32	50	39	440		
B7309	L	70	65	64	44	100	37	75	29	28	61	573	46·1	4
		31	51	65	100	37	114	42	124	58	48	670		
B7313	L	50	30	60	18	91	16	91	52	32	40	480	46·3	3
		23	60	39	87	57	91	31	57	38	73	556		
B7316	L	30	37	29	28	22	30	45	40	35	42	338	31·8	0
		59	58	95	72	82	92	66	81	44	77	726		
B7318	L	12	6	38	6	5	21	2	101	21	39	251	21·4	1
		80	113	89	57	105	104	116	83	79	94	920		
B7320	L	60	82	77	103	100	74	69	77	8	63	713	60·6	5
		27	10	28	20	26	71	90	91	15	85	463		
B7322	L	57	40	110	103	71	61	67	57	44	65	675	48·5	3
		57	69	48	66	65	108	83	105	53	71	725		
Ca diet Tot.		516	563	615	667	699	602	659	775·	300	622			
D. diet Tot.		630	750	894	919	817	1058	927	984	583	904			
Ca diet %		45·2	42·9	40·8	42·1	46·2	36·3	41·6	44·2	34·0	40·8			

Combined totals and percentage for Batches 1 + 2

Experimentals

Ca diet Tot;	794	1216	1219	1693	1335	1721	1532	1899	1687	1909
D. diet Tot.	825	559	607	277	439	244	419	270	213	135
Ca diet %	49·0	68·5	66·7	85·9	75·2	87·6	78·5	87·5	88·8	93·0

Controls

Ca diet Tot.	792	719	820	832	866	754	807	941	427	955
D. diet Tot;	1064	1303	1405	1497	1330	1674	1475	1531	1131	1248
Ca diet %	42·7	35·5	36·8	35·7	39·4	31·0	35·4	38·1	27·4	43·4

Table 4A

Selection of quinine-adulterated diet

Results of 3.1

Deprived (Quinine choice)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
D423	20	20	23	13	22	2	19	13	15	8	155	11·0	0
	86	110	121	155	128	146	138	128	103	140	1255		
D350	42	8	7	4	9	13	2	0	0	2	87	8·9	0
	35	63	64	91	102	100	113	114	92	119	893		
P1427	50	20	41	2	9	1	0	0	2	0	125	8·9	0
	51	103	96	158	133	154	132	165	107	161	1280		
P1415	36	20	16	37	52	29	53	27	31	52	353	21·4	0
	91	116	158	126	117	145	123	176	116	132	1300		
P1413	72	8	2	35	1	38	3	49	0	5	213	21·6	2
	99	63	56	2	34	5	142	113	94	163	771		
Ca diet	220	76	89	91	93	83	77	89	48	67			
Def d.	362	455	495	532	514	550	668	696	512	715			
Ca %	37·8	14·3	15·2	14·6	15·3	13·1	10·3	11·3	8·6	8·6			

Controls (Quinine choice)

P1453	88	16	22	20	2	3	3	0	1	2	157	9·9	0
	32	131	140	173	174	155	189	167	104	172	1437		
D418	36	23	10	28	5	2	2	0	0	1	107	10·0	0
	63	45	106	103	110	130	126	152	98	137	1070		
D420	31	64	75	64	57	42	33	21	2	30	419	28·1	0
	36	77	82	118	105	148	165	196	161	152	1240		
D417	16	21	9	8	6	8	10	0	1	1	80	5·5	0
	104	110	141	162	122	158	155	161	117	153	1383		
Ca diet	171	124	116	120	70	55	48	21	4	34			
Def d.	235	363	469	556	511	591	635	676	480	614			
Ca %	42·1	25·5	19·8	17·8	12·0	8·5	7·0	3·0	0·8	5·2			

Deprived (Plain choice)

P1440	25	57	46	52	43	66	61	63	23	70	506	58·3	7
	59	17	48	24	11	8	40	48	57	50	362		
P1418	2	67	62	44	72	55	69	87	56	69	583	52·8	7
	44	18	53	69	51	70	56	55	44	62	522		

[Contd.

Table 4A (contd.)

Deprived (contd)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
D413	96	65	41	96	30	43	81	130	45	133	760	58·4	7
	61	61	109	58	2	87	59	43	37	25	542		
P1409	93	53	95	91	87	75	83	84	79	88	828	55·4	7
	50	97	66	70	71	64	47	90	43	68	666		
C8706	1	1	90	87	108	40	96	81	74	97	675	51·7	6
	1	106	65	93	70	90	21	66	53	65	630		
Ca diet	217	243	334	370	340	279	390	445	277	457			
Def diet	215	299	341	314	205	319	223	302	234	270			
Ca %	50·2	44·8	49·5	54·1	62·4	46·7	63·6	59·6	54·2	63·7			

Controls (Plain choice)

C8101	22	25	34	51	87	68	53	110	34	84	568	42·9	3
	60	73	80	93	70	86	92	64	79	60	757		
P1422	60	98	101	118	121	143	135	148	111	105	1140	59·5	9
	66	50	81	89	71	80	89	93	59	98	776		
C8703	76	51	104	103	112	95	89	78	73	109	890	57·4	7
	26	84	67	56	65	64	71	103	56	68	660		
P1451	10	38	26	90	89	83	80	92	57	105	670	54·6	6½
	67	38	31	22	49	53	75	70	74	78	557		
Ca diet	168	212	265	362	409	389	357	428	275	403			
Def diet	219	245	259	260	255	283	327	330	268	304			
Ca %	44·6	46·4	50·6	58·2	61·6	57·9	52·2	56·5	50·6	56·7			

Results of 3.2 Table 5A

Deprived (Quinine choice)

D1029	30	14	12	14	12	34	4	4	15	1	140	21·4	1
	62	63	52	26	50	33	53	55	65	55	514		
D1041	24	72	5	10	3	27	0	12	1	16	170	22·2	1
	69	5	79	40	75	58	61	60	89	61	597		
D1022	25	46	12	54	36	95	17	76	35	77	473	57·8	5
	75	32	54	2	54	2	56	0	65	6	346		
D1025	36	116	12	145	11	120	3	106	4	110	663	59·0	5
	84	8	79	5	82	4	76	0	121	2	461		
D1038	10	89	7	90	20	37	10	30	15	34	342	34·0	2
	106	3	79	30	75	65	66	71	99	70	664		
Ca diet	125	337	48	313	82	313	34	228	70	238			
Def diet	396	111	343	103	336	162	312	186	439	194			
Ca %	24·0	75·2	12·3	75·2	19·6	65·9	9·8	55·1	13·8	55·1			

Table 5A (contd.)

Controls (Quinine choice)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
D1011	19	36	11	37	25	12	15	17	25	25	222	20·6	0
	92	79	94	115	80	79	65	91	88	71	854		
D1017	13	96	10	95	21	34	10	25	10	34	348	29·0	2
	127	5	118	37	106	89	82	85	113	92	854		
D1016	31	40	30	15	40	38	20	30	18	45	307	32·5	1
	59	65	58	102	57	75	34	9	127	52	638		
D1021	3	70	9	75	15	54	4	46	19	61	356	34·2	4
	104	38	82	49	100	45	79	50	96	41	684		
P1441	121	115	59	53	102	48	66	80	88	70	802	46·7	2
	80	74	100	163	36	97	72	92	93	107	914		
Total Ca diet	187	357	119	275	203	186	115	198	160	235	213		
Def diet	462	261	452	379	385	332	327	517	363	470			
Ca %	28·8	57·8	20·8	37·1	34·9	32·6	25·7	23·6	39·3	31·2			

Deprived (Plain choice)

D1024	93	69	129	155	4	121	1	125	94	105	896	73·5	7
	47	64	2	0	96	1	73	0	40	0	323		
P1429	36	154	132	207	135	116	126	145	161	156	1368	84·4	9
	83	43	20	9	21	49	1	25	2	0	253		
D1033	41	16	41	80	85	110	62	143	76	105	759	73·9	7
	52	46	49	29	31	11	16	6	26	2	268		
D1019	79	165	149	190	150	142	122	150	170	155	1472	93·7	9
	66	5	1	1	2	7	10	2	0	5	99		
D1036	35	143	101	152	125	127	81	140	98	150	1152	89·0	9
	53	3	18	5	6	3	13	0	37	5	143		
Total Ca diet	284	547	552	784	499	616	392	703	599	671			
Def diet	301	161	90	44	156	71	113	33	105	12			
Ca %	48·5	77·3	86·0	94·7	76·2	89·7	77·6	95·5	85·1	98·2			

Controls (Plain choice)

D1027	62	73	154	10	90	157	54	160	156	145	1061	69·1	7
	58	94	62	196	8	15	41	0	0	0	474		
D1026	124	70	81	53	61	59	66	35	89	73	711	52·2	5
	47	72	56	125	62	71	31	84	46	57	651		

[Contd.

Table 5A (contd.)

Controls (Plain choice) (contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
P1439	2	128	1	163	2	156	26	130	53	130	791	52·8	5
	99	1	137	40	150	4	114	31	105	26	707		
D1018	36	37	89	101	74	82	77	97	106	95	794	79·6	8
	67	74	1	27	13	15	3	1	2	0	203		
P1412	89	40	100	103	110	3	72	1	142	3	663	56·2	5
	62	85	21	27	3	96	0	117	1	105	517		
Totals													
Ca diet	313	348	425	430	337	451	295	423	546	446			
Def diet	333	326	277	415	236	201	189	233	154	188			
Ca %	48·5	51·6	60·5	50·9	58·8	69·2	61·0	64·5	78·0	46·6			

Table 6A

Results of Experiment 4.1.

Selection of 2% calcium carbonate in mash

In each case the calcium-supplemented diet is given first, and the deficient diet second.

Bird No.	Mar 17	18	19	20	21	22	23	24	25	26	Tot.	Ca %	Ca No.
Deprived													
B4928	22	39	48	15	22	29	20	47	25	64	331	40·1	2
L	57	42	55	38	59	48	74	28	56	20	477		
B4931	33	11	67	20	45	27	53	49	66	40	411	51·3	5
L	10	75	34	59	33	52	37	26	16	48	390		
B4936	43	48	40	38	46	54	46	63	51	51	480	48·8	5
R	58	41	69	34	61	45	60	39	44	52	503		
B4937	58	43	38	43	63	78	89	64	52	78	606	64·2	6½
L	42	31	52	43	31	26	25	16	63	9	338		
B5050	73	68	44	61	53	50	50	82	59	65	605	55·6	5
R	12	20	85	45	60	55	70	28	49	59	483		
B5051	41	20	40	55	40	41	61	74	68	70	510	47·1	4
R	49	72	62	52	55	62	69	51	53	47	572		
Total Ca diet	270	229	277	232	269	279	319	379	321	368			
Def diet	228	281	357	271	299	288	335	188	281	235			
Ca %	54·2	44·9	43·7	46·1	47·3	49·2	48·8	66·8	53·3	61·0			

NOTE: L = calcium diet initially on left
R = calcium diet initially on right

Table 6A (contd.)

Normal Controls

Bird No.	Mar 17	18	19	20	21	22	23	24	25	26	Tot.	Ca %	Ca No.
B4929	113	50	110	71	83	41	89	71	58	75	761	51·6	4
R	30	63	60	55	77	82	85	86	99	78	715		
B4933	7	24	45	45	55	37	81	47	65	52	458	40·9	2
L	86	72	73	63	66	59	53	56	62	72	662		
B4934	32	47	76	51	53	61	44	44	47	66	521	49·5	5
R	61	40	51	50	55	21	80	62	64	48	532		
B4935	50	71	51	73	69	53	48	52	62	39	568	49·3	2½
L	61	25	59	33	69	59	70	59	63	87	585		
B5042	1	88	66	52	60	70	59	53	88	42	579	51·3	6
L	80	27	64	50	65	31	72	49	31	81	550		
B5045	6	66	68	57	69	45	52	46	69	62	540	52·2	8
R	61	63	64	51	57	47	25	42	51	34	495		
B5046	48	42	73	66	52	54	66	37	45	56	539	42·6	½
R	47	54	76	74	90	68	66	72	99	79	725		
B5054	30	42	96	48	56	46	54	47	46	48	513	50·1	2
R	25	55	5	54	62	52	60	44	83	70	510		
B5057	47	44	70	45	58	23	68	26	33	32	446	43·3	2
L	25	35	73	58	67	65	47	67	65	81	583		

Totals

Ca diet	334	474	655	508	555	430	561	423	513	472
Def diet	476	434	525	488	608	484	558	537	617	630
Ca %	41·2	52·2	55·5	51·0	47·7	47·0	50·1	44·1	45·4	42·8

Table 7A

Results of Experiment 4.2

Selection of visually identical diets

Bird No.	Aug. 24	25	26	27	28	29	30	31	Sep 1	2	Tot.	Ca %	Ca No.
Deprived													
B7790	40	86	83	103	72	98	62	36	51	29	660	58·4	7
L	22	5	75	32	49	73	55	72	49	38	470		
B7430	39	76	70	87	88	70	53	66	55	64	668	71·8	9
L	13	4	27	2	1	61	44	43	48	19	262		
B7849	40	64	100	126	103	112	65	61	58	99	828	73·3	9
L	41	58	35	4	7	45	26	32	49	4	301		

NOTE: L = calcium diet initially on left
R = calcium diet initially on right

[contd.

Table 7A (Contd.)

Deprived (contd.)

Bird No.	Aug. 24	25	26	27	28	29	30	31	Sept. 1	2	Tot	Ca %	Ca No.
B7853	80	50	107	82	97	59	76	61	48	58	718	55·7	6
L	19	43	38	55	40	104	69	64	84	54	570		
B7789	34	49	80	90	74	58	64	74	75	81	679	75·5	8½
L	11	22	14	0	16	58	23	28	37	11	220		
B7857	5	35	17	57	34	56	44	70	27	65	410	55·0	6
R	16	32	22	21	33	58	32	43	54	25	336		
B7853	48	87	67	99	91	98	42	31	49	68	680	66·1	7
R	36	24	28	22	15	60	38	53	49	23	348		
B7858	45	72	77	97	91	111	18	96	92	90	789	83·8	8
R	6	2	18	0	0	10	64	17	35	0	152		
B7788	74	131	138	60	84	115	76	85	96	51	910	56·6	4
R	42	36	5	64	71	109	88	94	97	91	697		
B7784	46	66	86	92	71	97	65	75	96	74	768	82·6	9
R	19	7	20	6	10	45	22	19	8	6	162		
B7783	50	70	95	79	86	105	87	67	80	66	785	76·9	9
R	13	23	7	16	0	45	20	47	35	30	236		
Totals													
Ca diet	501	786	920	972	891	979	652	722	727	745			
Def diet	238	256	289	222	242	668	481	512	545	301			
Ca %	67·8	75·5	76·2	81·4	78·6	59·5	57·6	58·5	57·2	71·2			

Normal Controls

B7789	50	80	84	125	52	107	113	42	42	60	755	60·8	6
L	45	28	40	30	42	75	54	51	52	70	487		
B7848	72	70	104	73	107	70	123	109	118	78	924	68·5	7
L	50	56	41	81	21	123	0	15	14	24	425		
B7780	106	57	65	86	96	78	91	51	63	22	715	46·0	2
L	19	115	100	117	20	108	75	97	103	81	841		
B7781	107	75	110	76	87	113	81	109	108	48	914	49·6	4
L	75	73	73	70	97	129	121	79	113	99	929		
B7786	110	57	127	84	91	141	91	59	81	25	866	55·8	5
L	33	65	30	70	43	126	72	91	86	70	686		
B7844	66	97	93	110	94	117	86	85	87	75	910	50·3	5
R	51	71	90	62	73	136	105	119	120	71	898		

NOTE: L = calcium diet initially on left
R = calcium diet initially on right

[Contd.

Table 7A (contd.)

Normal Controls (contd.)

Bird No.	Aug 24	25	26	27	28	29	30	31	Sept. 1	2	Tot.	Ca %	Ca No.
B7859	86	112	127	96	83	122	125	59	76	55	941	48·1	4
R	80	96	105	95	90	150	85	120	91	104	1016		
B7846	35	63	85	75	77	91	66	97	52	44	685	56·6	6
R	52	34	55	42	36	105	54	21	77	48	524		
B7787	74	73	66	72	61	93	79	73	75	74	740	50·7	4
R	62	46	83	74	72	110	71	79	72	50	719		
B7854	75	113	130	115	102	112	105	72	94	42	960	53·8	6
R	60	52	73	56	79	132	91	100	90	92	825		
Totals													
Ca diet	781	797	991	912	850	1044	960	756	796	523			
Def diet	527	636	696	697	573	1194	728	772	818	709			
Ca %	59·8	55·6	58·8	56·8	59·7	46·7	56·9	49·6	49·3	41·4			

Note: L = calcium diet initially on left
R = calcium diet initially on right

Table 8A

Results of Experiment 5.1. Selection of 2% Strontium Carbonate

Experimental Birds (calcium deprived)

In each case the strontium-supplemented diet is given first, and the deficient diet second.

Bird No,	Jul 18	19	20	21	22	23	24	25	26	27	Tot.	Sr %	Sr No.
B7317	37	30	47	65	37	50	64	52	44	58	484	73·6	8
L	23	37	14	11	23	15	24	12	10	4	173		
B7304	36	74	60	24	0	1	0	10	0	2	207	20·9	2
L	42	39	27	86	89	99	135	80	89	96	782		
B7315	21	80	83	94	89	74	51	62	39	80	673	85·7	9
L	43	11	7	5	0	0	25	0	21	0	112		
B7252	44	34	35	20	40	32	65	35	36	35	376	54·6	6
R	21	46	41	56	23	27	23	34	19	22	312		
B7308	30	68	39	67	34	52	24	50	31	65	460	48·4	5
R	71	58	53	19	51	31	93	42	52	20	490		
B7307	47	46	42	37	46	43	39	17	14	10	341	48·6	3½
R	24	59	42	57	43	35	41	33	10	16	360		

NOTE: L = Strontium diet initially on left
R = Strontium diet initially on right

[Contd.

Table 8A (Contd.)

Experimental Birds (Calcium deprived)(contd.)

Bird No.	Jul 18	19	20	21	22	23	24	25	26	27	Tot.	Sr %	Sr No.
B7310	32	41	57	24	48	2	70	1	37	0	312	60·0	5
R	0	25	3	31	0	37	0	60	0	52	208		
Totals													
Sr diet	247	373	363	331	294	254	313	227	201	250			
Def diet	224	275	187	265	229	244	341	261	201	210			
Sr %	52·4	57·6	66·0	55·5	56·2	51·0	47·9	46·5	50·0	54·3			
Normal Control Birds													
B7301	69	95	129	110	84	90	104	74	73	78	906	66·3	9
L	6	54	16	51	75	49	63	36	53	58	461		
B7250	73	50	93	75	85	42	93	60	85	47	703	51·7	4
L	36	95	59	80	50	91	61	63	35	88	657		
B7251	22	73	40	52	62	41	48	84	43	41	506	42·3	2
L	42	47	81	76	73	80	89	77	60	66	691		
B7248	56	86	66	58	90	48	85	98	103	72	762	55·1	5
L	13	22	28	82	63	85	94	112	55	68	622		
B7321	50	80	15	100	34	100	106	93	29	95	702	70·5	6
R	26	27	67	7	69	5	16	3	62	12	294		
B7257	57	109	94	108	104	99	113	96	90	111	981	92·0	9
R	30	6	14	6	4	2	11	2	10	0	85		
B7254	12	11	66	84	49	67	65	67	54	79	554	48·2	6
R	61	67	61	49	89	52	74	48	40	54	595		
Totals													
Sr diet	339	504	503	587	508	487	614	572	477	523			
Def diet	214	318	326	351	423	364	408	341	315	346			
Sr %	61·3	61·3	60·6	62·5	54·6	57·2	60·1	62·7	60·2	60·2			

Table 9A

Results of Experiment 5.2 Selection of 4% Strontium carbonate

Deprived

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Sr %	Sr No.
C9808	61	59	31	60	0	33	0	67	23	38	372	40·2	3
	50	21	62	2	103	58	64	9	107	77	553		

NOTE: L = Strontium diet initially on left
R = Strontium diet initially on right

[Contd.

Table 9A (contd.)

Deprived (contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Sr %	Sr No.
C9807	22	49	66	7	84	5	86	2	77	1	399	42·0	4
	50	56	41	126	20	35	0	85	1	137	551		
C9798	36	39	24	50	30	44	3	34	31	55	346	47·6	5
	54	19	36	32	48	11	46	30	66	39	381		
C9818	70	17	72	65	54	85	14	81	15	80	553	45·2	3
	27	87	48	74	71	20	85	32	138	89	671		
C9813	21	73	75	83	86	63	75	80	52	94	702	44·4	4
	28	82	91	119	84	98	73	69	166	69	879		
C9805	46	43	53	47	58	31	59	27	47	33	444	41·9	2
	43	62	55	94	47	53	30	70	83	80	617		
C9811	98	23	99	65	98	80	55	37	125	18	698	52·0	5
	35	104	46	85	45	33	43	85	36	133	645		
C9799	19	80	61	91	56	79	44	40	72	78	620	47·2	3
	42	58	99	60	81	48	73	67	87	80	695		
Totals													
Sr diet	373	383	481	468	468	420	336	368	442	397			
Def diet	329	489	478	592	499	356	414	447	684	704			
Sr %	53·1	43·9	50·2	44·2	48·4	54·1	44·8	45·2	39·3	36·1			
Sr No.	4	3	3	3	4	4	4	4	2	2			
Controls													
C9800	32	16	11	73	55	58	46	60	86	69	506	52·4	5
	29	33	37	50	56	30	36	39	56	94	460		
C9806	1	64	38	70	38	60	3	105	8	104	491	44·8	5
	33	10	67	68	84	32	99	25	125	62	605		
C9809	12	86	1	115	2	79	6	68	66	47	482	33·7	3
	85	48	157	20	118	28	117	87	119	170	949		
C9812	21	105	26	114	79	22	91	6	69	121	654	52·9	5
	60	38	97	41	49	78	34	64	110	12	583		
C9802	63	56	77	21	117	2	90	1	77	5	509	48·3	5
	19	49	40	137	7	96	5	85	11	95	544		
C9801	96	9	92	90	84	52	96	22	162	32	735	50·9	4
	65	94	66	105	50	53	0	112	7	158	710		
C9796	0	14	77	17	91	41	104	2	69	35	450	41·3	3
	0	60	17	116	46	75	20	102	84	120	640		
Totals													
Sr diet	225	350	322	500	466	314	436	264	537	413			
Def diet	291	332	481	537	410	392	311	514	512	711			
Sr%	43·6	51·3	40·1	48·2	53·2	44·5	58·4	33·9	51·2	36·7			
Sr No.	3½	4	3	4	4	3	5	2	3	2			

Table 10A

Deprived

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Sr %	Sr No.
D100	11	34	0	2	0	4	25	22	1	31	130	10·6	0
	63	94	111	83	147	140	119	115	124	103	1099		
D97	77	73	58	70	32	81	17	66	20	107	601	37·5	3
	112	127	135	107	54	36	150	64	125	92	1002		
D91	48	3	14	5	2	5	28	9	5	11	130	10·9	0
	39	141	110	115	116	109	86	113	95	134	1058		
D88	84	65	176	61	66	86	51	52	81	82	804	39·3	2
	122	90	141	132	162	124	139	154	98	80	1242		
D89	67	53	46	22	26	9	6	9	11	7	256	16·9	0
	39	105	101	121	140	138	153	156	135	166	1254		
D95	75	77	23	35	46	26	85	46	34	23	470	28·6	0
	57	108	134	117	140	134	92	109	134	147	1172		
D94	0	3	37	7	4	2	16	25	41	16	151	14·8	0
	78	111	82	83	96	90	89	87	51	100	867		
D93	70	94	102	107	99	6	30	69	89	48	714	38·8	3
	13	77	112	86	127	146	162	165	88	150	1126		
Totals													
Sr diet	432	402	456	309	275	219	258	299	282	325			
Def diet	523	853	926	844	982	917	990	963	850	972			
Sr %	45·2	32·3	33·0	26·8	21·9	19·3	20·7	23·7	24·9	25·1			
Sr No.	4	1	1	1	0	1	0	1	1	2			
Controls													
D84	76	76	53	79	52	61	48	45	79	34	603	46·5	3
	16	22	77	67	98	71	112	59	49	123	694		
D90	46	55	45	31	41	108	60	107	68	103	664	38·9	3
	44	90	123	120	141	75	111	117	127	95	1043		
D78	56	55	79	50	47	30	69	22	87	29	524	35·2	1
	87	97	83	100	96	116	99	138	4	145	965		
D83	83	71	101	86	60	95	42	84	67	71	760	53·7	4
	37	54	53	35	86	38	116	59	71	105	654		
D87	77	65	57	52	43	19	122	43	46	21	545	37·0	1
	71	97	122	119	88	108	41	60	83	139	928		
D86	46	46	77	76	77	71	92	79	76	68	708	51·9	5
	30	61	35	65	94	90	88	74	45	73	655		

[Contd.

Table 10A (contd.)

Controls (Contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Sr %	Sr No.
D81	11	21	46	41	73	51	63	61	44	55	466	30·8	0
	91	116	81	106	103	110	102	115	101	122	1047		
D79	71	108	30	113	98	108	61	50	2	61	702	43·7	3
	100	125	99	1	73	35	133	83	143	111	903		
D80	37	39	42	9	19	13	17	37	26	39	278	28·4	0
	29	53	72	100	66	23	70	83	95	111	702		
D96	3	85	34	109	46	90	61	51	58	108	645	43·0	3
	104	103	119	71	106	3	88	108	63	89	854		
Totals													
Sr diet	506	621	564	646	556	646	635	579	553	589			
Def diet	609	818	864	784	951	669	960	896	781	1113			
Sr %	45·4	43·2	39·5	45·2	36·9	49·1	39·8	39·3	41·5	34·6			
Sr No.	6	2	2	5	1	4	2	2	3	2			

Table 11A

Results of Experiment 6
Discrimination between calcium and strontium

In each case the calcium-supplement diet is given first, and the strontium diet second.

Deprived												Ca %	Ca No.
B7421	40	57	88	55	51	70	64	76	83	87	671	56·3	5½
L	61	64	33	55	55	32	76	44	57	44	521		
B7434	102	20	94	72	87	110	113	101	94	87	880	62·1	8
L	45	109	51	62	27	9	60	42	67	65	537		
B7855	27	64	74	94	52	83	67	72	80	70	683	56·7	8
L	55	52	50	32	62	40	65	62	42	61	521		
B7414	142	81	49	45	53	64	109	88	128	104	863	58·3	5
L	32	85	25	42	70	62	40	91	53	117	617		
B7426	1	0	17	34	52	53	73	45	34	36	345	31·0	1
L	135	43	77	33	62	55	95	94	52	122	768		
B7418	86	46	77	56	50	66	90	70	114	82	737	55·7	5
R	58	79	71	58	53	49	65	81	17	55	586		
B7422	67	20	116	85	123	82	146	89	78	100	906	68·1	8
R	35	84	25	12	19	24	12	80	73	61	425		

NOTE: R = Calcium diet initially on right
L = Calcium diet initially on left

[Contd.

Table 11A (Contd.)

Deprived (Contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Total	Ca %	Ca No.
B7433	122	21	67	34	20	30	40	103	56	56	549	54·2	4
R	0	36	59	45	42	43	80	72	35	51	463		
B7431	46	54	71	58	52	45	91	60	84	72	633	50·1	3
R	45	77	65	61	63	47	56	73	65	76	628		
B8169	26	55	55	57	41	56	36	62	28	59	475	46·9	4
	41	62	80	26	65	45	69	41	66	42	537		
B8164	51	51	63	76	56	95	70	68	28	63	621	47·1	4
	43	84	73	54	103	61	77	63	82	58	698		
B8168	11	58	39	93	1	106	0	95	10	66	479	42·1	4
	51	75	83	17	119	22	117	32	103	41	660		
B8691	35	73	61	42	35	48	50	45	56	82	527	47·1	2
	27	80	39	75	65	59	52	48	53	93	591		
B8689	39	86	15	96	31	69	34	54	22	80	526	51·2	5
	39	64	56	7	67	39	60	31	67	72	502		
P1128	5	57	0	61	0	23	20	41	46	96	349	46·8	4
	56	64	36	2	70	47	32	27	27	35	396		
B8684	73	57	46	57	50	81	87	60	86	80	677	57·5	5½
	69	65	50	56	50	37	29	43	15	87	501		
B8167	34	50	46	62	66	87	59	40	57	55	556	54·4	5
	1	67	36	35	32	18	73	51	54	99	466		
B8176	18	98	87	90	99	86	103	71	98	124	874	51·7	6
	52	108	71	82	76	92	90	96	63	86	816		
B8171	2	74	24	73	69	63	36	57	51	89	538	51·9	6
	1	61	60	39	61	37	57	27	62	93	498		
Totals													
Ca diet	964	1106	1155	1303	1021	1389	1298	1297	1233	1488			
Sr diet	896	1406	1132	855	1230	852	1221	1098	1069	1342			
Ca %	51·8	44·0	50·5	60·4	45·4	62·0	51·5	54·1	53·6	52·6			
Ca No.	11½	3	11	14½	5½	13	8	12	13	12			

NOTE: R = Calcium diet initially on right
L = Calcium diet initially on left

Table 11A (Contd.)

Normal Controls

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
B7427	14	137	47	133	20	125	146	25	132	30	809	52·3	5
	114	9	133	28	119	5	40	138	27	124	737		
B7843	68	57	80	67	59	70	57	81	51	55	645	46·7	4
	115	76	96	42	55	37	98	56	88	73	736		
B7424	47	49	61	72	84	55	113	80	100	58	719	57·2	6
	70	54	76	40	29	46	32	74	54	62	537		
B7420	60	49	109	52	82	45	115	84	95	43	734	53·6	5
	48	60	45	70	58	82	54	81	61	76	635		
B7429	94	74	90	91	91	78	76	78	31	108	811	55·1	7
	89	80	83	69	58	35	61	37	96	54	662		
B7425	109	103	112	87	87	47	130	78	98	118	969	58·4	6
	28	58	70	73	58	75	57	81	124	62	686		
B7428	102	128	114	114	93	77	129	119	112	96	1084	55·1	5
	52	62	125	76	129	114	63	125	59	78	883		
B7412	7	91	16	100	34	75	50	110	108	98	689	56·2	6
	77	4	115	17	59	0	126	26	56	56	536		
B7423	72	84	117	71	108	87	138	102	110	95	984	55·8	8
	26	45	74	92	67	83	114	101	106	71	779		
B8175	38	17	0	20	82	78	76	84	69	88	552	37·4	1
	32	112	146	117	93	82	91	85	94	70	922		
B8174	50	70	83	79	96	89	105	105	97	78	852	51·3	5
	58	72	90	81	73	95	90	87	86	77	809		
B8161	35	55	72	51	68	74	96	53	74	68	646	51·6	5
	10	62	51	62	59	59	74	65	100	63	605		
B8170	32	7	73	29	66	64	71	69	48	73	532	48·5	6
	20	63	51	79	62	46	60	53	73	59	566		
B9098	17	153	78	102	88	84	108	68	60	105	863	46·5	4
	116	112	93	95	109	106	89	84	98	92	994		
B8165	143	115	59	149	104	41	78	99	62	145	995	52·8	4
	35	144	84	84	126	122	70	34	95	97	891		
B8166	33	75	82	64	45	103	126	99	71	101	799	48·4	3
	34	140	85	138	89	61	49	58	95	102	851		
B8173	73	105	63	82	78	86	78	87	86	109	847	51·6	5
	47	87	73	70	92	92	81	78	73	100	793		
B8930	55	106	71	87	71	80	70	80	66	96	782	52·3	6
	56	99	60	70	72	62	77	56	73	87	712		

[Contd.

Table 11A (Contd.)

Normal Controls (Contd.)

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
B8933	57	86	64	61	83	47	92	59	79	79	704	50·8	4
	30	88	51	75	65	70	58	81	69	96	683		
B8172	9	89	49	76	94	65	89	12	35	54	572	41·0	3
	40	97	76	97	80	62	77	96	61	136	822		
B8163	70	147	73	127	82	31	82	51	72	137	872	51·6	5
	62	144	70	77	61	74	117	67	85	60	817		
Totals													
Ca diet	1185	1797	1513	1714	1615	1501	2025	1620	1656	1834			
Sr diet	1159	1688	1747	1551	1613	1408	1583	1563	1673	1695			
Ca %	50·6	51·9	46·4	52·5	50·0	51·6	56·1	50·9	49·7	52·0			
Ca No.	12	9	9	12	12	11	15	12	9	14			

Table 12.1A

Results of Experiment 7. Discrimination between calcium and strontium

In each case the calcium-supplement diet is given first, and the strontium diet second.

Bird No.	Oct 24	25	26	27	28	29	30	31	Nov 1	2	3	Tot.	Ca %	Ca No.
Deprived														
P1132	5	10	98	104	69	64	51	60	92	31	98	682	53·2	6
	3	4	62	55	98	92	48	42	41	72	82	599		
P1137	55	73	82	48	38	43	36	40	79	72	81	647	65·1	9
	21	20	32	42	34	23	22	29	5	52	67	347		
P1138	23	5	0	82	41	82	54	68	41	67	67	530	67·9	7½
	19	0	28	0	22	13	28	44	41	17	38	250		
B8683	75	18	95	94	102	104	86	83	75	79	114	925	60·6	8
	70	78	61	53	37	58	34	40	62	43	66	602		
B8689	6	40	102	95	84	134	90	116	103	121	117	1008	73·7	9
	7	2	69	76	31	24	54	7	20	0	70	360		
P1141	0	58	96	78	75	38	31	28	61	92	125	682	63·4	7½
	0	0	23	67	25	38	32	21	52	53	82	393		
P1142	60	42	80	104	46	30	33	45	46	52	69	607	57·3	6
	18	37	35	50	24	38	24	40	57	65	64	452		
B8692	91	71	61	76	86	85	70	63	83	73	106	865	61·0	8
	15	40	76	65	40	64	43	57	54	43	56	553		
B8665	98	94	93	83	39	70	74	76	72	72	92	863	56·5	6
	48	48	84	87	74	71	39	45	47	46	74	663		
P1127	31	58	61	69	52	64	53	49	56	15	63	571	78·4	8
	16	3	21	21	9	15	7	2	2	36	25	157		
B 8694	33	47	104	76	72	52	86	47	82	35	109	743	62·3	5
	22	34	12	78	22	70	16	64	33	63	36	450		

[Contd.

Table 12.1A (contd.)

Bird No.	Oct 24	25	26	27	28	29	30	31	Nov 1	2	3	Tot.	Ca %	Ca No.
Deprived (Contd.)														
B8695	22	58	50	57	44	68	55	13	74	59	86	586	56·6	8
	28	7	34	48	37	54	48	63	40	36	54	449		
Totals														
Ca diet	499	574	922	966	748	834	719	688	864	768	1127			
Sr diet	267	273	537	642	453	560	395	454	454	526	714			
Ca %	65·1	67·8	63·2	60·2	62·3	59·8	64·6	60·2	65·5	59·3	61·2			
Ca No.	9½	11	10	10	10	7½	11	10	10½	8	12			
Controls														
B8688	24	67	76	71	100	99	53	103	68	97	93	851	50·2	4
	30	47	36	77	103	100	101	66	104	67	114	845		
B8687	0	84	46	71	47	78	46	48	69	60	93	642	50·3	4
	0	31	81	63	59	70	59	63	62	68	79	635		
P1136	0	0	83	38	111	43	117	29	126	21	121	689	48·0	4½
	0	0	71	135	19	130	8	122	25	127	108	745		
P1134	0	10	75	77	88	57	70	60	26	17	62	542	57·7	5
	0	5	78	67	0	76	36	44	43	45	4	398		
B8666	0	85	75	91	41	55	46	63	40	84	61	641	51·8	4
	2	46	81	63	57	59	77	30	65	26	91	597		
P1140	0	0	93	94	84	102	90	98	77	99	111	848	53·0	5½
	1	0	83	95	57	109	82	58	104	41	122	752		
B8686	1	71	54	60	60	48	46	51	52	48	96	587	50·5	4
	12	39	55	38	67	57	43	48	63	51	103	576		
B8693	25	42	68	66	75	82	82	60	72	65	82	719	54·6	7
	30	47	52	79	53	73	50	38	44	36	95	597		

[Contd.

Table 12.1A (contd.)

Bird No.	Oct 24	25	26	27	28	29	30	31	Nov 1	2	3	Tot.	Ca %	Ca No.
Controls (Contd.)														
P1133	41	34	73	65	35	69	42	32	43	34	57	525	54·5	7
	27	36	66	54	33	38	32	26	30	39	58	439		
B8667	0	75	61	79	38	54	49	64	37	42	78	577	44·2	4
	83	62	51	74	68	64	60	39	73	76	77	727		
P1135	18	8	76	92	50	112	62	79	54	85	117	753	48·5	5
	22	31	67	74	81	84	94	78	105	77	85	798		
B8690	46	92	86	89	66	85	78	71	41	78	73	805	51·4	6
	48	65	60	62	57	72	65	78	75	88	92	762		
Totals														
Ca diet	155	568	866	893	795	884	781	758	705	730	1044			
Sr diet	255	409	781	881	654	932	707	690	793	741	1028			
Ca %	37·9	58·2	52·7	50·3	54·9	48·7	52·6	52·3	47·1	49·7	50·5			
Ca No.	2½	8	8	8	6	5	7	9	4	5	5			

After reversal of food dye

In each case strontium diets are given first - the red food dye is now present in the calcium carbonate diets.

Deprived Bird No.	Nov. 4	5	6	7	8	9	10	Total	Ca %	Ca No.
P1132	71	23	107	19	128	40	67	455	55·1	5
	40	121	16	65	135	110	72	559		
P1137	158	111	117	124	139	113	120	882	3·0	0
	0	0	0	24	1	2	0	27		
P1138	44	74	52	92	48	76	39	425	40·6	2
	42	9	36	35	60	41	68	291		

[Contd.

Table 12.1A (Contd.)

Deprived (contd.)

Bird No. Nov.	4	5	6	7	8	9	10	Total	Ca %	Ca No.
B8683	102	96	82	64	82	111	80	617	40·5	1½
	38	20	42	113	76	51	80	420		
B8689	43	127	36	104	52	118	52	532	47·2	4
	67	4	89	77	98	40	100	475		
P1141	107	110	123	127	119	86	156	828	39·6	1
	34	37	66	124	103	130	49	543		
P1142	90	69	89	80	86	63	83	560	38·2	1
	23	32	24	74	56	87	50	346		
B8692	118	118	88	55	27	76	15	497	49·5	4
	10	11	41	89	122	87	127	487		
B8665	96	96	78	93	76	114	44	597	47·1	3
	35	60	71	108	90	79	89	532		
P1127	31	21	21	31	46	51	55	256	58·3	4
	36	58	66	62	44	48	44	358		
B8694	72	56	100	60	83	47	73	491	41·5	2½
	42	56	17	82	34	89	28	348		
B8695	75	67	64	47	36	78	39	406	46·9	3
	31	22	48	89	71	33	65	359		
Totals										
Sr diet	1007	968	957	896	922	973	823			
Ca diet	398	430	516	942	890	797	772			
Ca %	28·3	30·7	35·0	51·2	49·2	45·0	48·4			
Ca No.	2	2½	2	7	6	5	6½			

Table 12.1A (contd)

Controls Bird No.	Nov. 4	5	6	7	8	Total	Ca %	Ca No.
B8688	139 128	65 139	127 93	140 146	129 109	600 615	50·6	2
B8687	107 53	110 47	67 72	85 66	74 61	443 299	40·3	1
P1136	134 64	46 114	131 51	77 137	126 62	514 428	45·4	2
P1134	65 151	72 71	74 81	128 112	119 122	458 537	54·0	3
B8666	87 100	86 66	65 91	77 113	69 95	384 465	54·8	4
P1140	50 61	47 83	13 114	42 125	12 117	164 500	75·3	5
B8686	87 48	82 69	66 73	99 128	74 112	408 430	51·3	3
B8693	50 25	34 48	69 57	49 64	62 40	264 234	47·0	2
P1133	22 153	88 25	81 99	105 85	47 105	343 467	57·7	3
P1135	66 78	104 79	100 100	107 124	108 84	485 465	48·9	2½
B8690	72 82	136 134	114 106	158 127	118 107	598 556	48·2	1
Totals Sr diet	879	870	907	1067	938			
Ca diet	943	875	937	1227	1014			
Ca %	51·7	50·1	50·7	53·5	51·9			
Ca No.	6	4	6½	7	5			

Table 12.2A

Plasma Calcium levels (Nov.9)

Experimental		Control	
P1132	10·6	B8688	11·1
P1138	10·4	B8687	11·3
B8683	10·1	B8666	11·3
P1142	10·4	P1140	12·0
B8665	10·2	P1133	11·1
B8684	10·8	P1135	11·3

Table 22A

Results of Experiment 10.1

Selection of calcium after exclusive exposure for 1 day

In each case the calcium-supplemented diet is given first, and the deficient diet second.

Bird No.	Day 0	1	2	3	4	5	6	7	8	9	Tot.	Ca %	Ca No,
Deprived Group													
C1161	130	77	31	78	72	54	83	56	56	59	566	56·1	6
L		22	79	35	35	69	36	50	49	68	443		
C1386	109	72	70	71	78	28	70	79	67	75	610	56·0	7
L		67	75	68	37	43	37	44	47	62	480		
C1390	60	34	54	65	60	64	76	72	71	62	558	70·5	9
L		30	23	9	35	23	24	34	15	41	234		
P1298	48	98	77	111	93	86	90	87	95	105	842	58·1	9
R		40	56	49	90	63	84	83	63	79	607		
C1354	176	113	128	102	171	65	99	71	168	101	1018	55·5	5
R		98	84	126	13	97	91	151	26	129	815		
C1509	151	114	132	113	129	107	121	101	124	116	1057	70·4	9
R		57	31	54	27	40	73	58	33	71	444		
Totals													
Ca diet	674	508	492	540	603	404	539	466	581	518			
Def diet		314	348	341	237	335	345	420	233	450			
Ca %		61·8	58·6	61·3	71·8	54·7	61·0	52·6	71·4	53·5			
Ca No.		6	4	5	6	3	6	5	6	4			

NOTE: R = calcium diet initially on right
L = calcium diet initially on left

Table 22A (contd.)

Bird No.	Day 0	1	2	3	4	5	6	7	8	9	Tot.	Ca %	Ca No.
Cl352		106	126	105	125	53	136	57	33	123	864	55·2	4½
L	154	112	126	96	10	61	56	80	94	66	701		
Pl294		136	119	153	146	74	147	105	85	86	1051	59·5	8
L	174	103	103	116	61	39	59	97	14	122	714		
Cl520		145	115	101	76	85	131	47	157	49	906	60·9	7
L	101	70	71	88	57	69	44	91	4	88	582		
Cl307		117	114	112	115	87	110	88	40	72	855	83·5	8
R	121	27	1	4	2	0	10	15	57	53	169		
Cl392		140	145	110	147	106	176	102	100	122	1148	64·5	8
R	178	145	43	65	34	65	30	82	73	94	631		
Cl358		73	32	74	72	72	74	23	50	33	503	63·4	6
R	37	35	0	11	15	14	23	69	57	67	291		
Totals													
Ca diet		717	651	655	681	477	774	422	465	485			
Def diet	765	492	344	380	179	248	222	434	299	490			
Ca %		59·3	65·4	63·2	79·2	65·8	77·7	49·3	60·9	49·7			
Ca No.		4	5½	6	6	5	6	3	3	3			
Controls													
Cl395	119	81	99	81	63	102	104	64	69	110	773	48·0	4
L		74	75	100	119	66	99	78	114	112	837		
Cl355	143	146	110	140	101	75	103	99	85	102	961	53·0	4
L		58	119	75	102	99	118	91	96	95	853		
Cl360	115	58	53	71	87	83	90	61	48	69	620	46·5	2
L		84	92	75	49	44	93	102	75	100	714		
Cl391	138	104	41	146	58	77	66	82	53	74	711	55·7	5
R		3	94	23	80	64	74	69	98	61	566		
Cl357	146	71	141	92	128	53	149	88	92	59	873	49·8	4
R		144	53	104	68	114	65	132	74	126	880		
Cl351	129	79	106	91	26	113	97	66	75	89	742	67·7	8
R		23	26	30	95	8	51	55	39	27	354		
Totals													
Ca diet	790	539	550	621	463	503	609	460	422	503			
Def."		386	459	407	513	395	500	527	496	521			
Ca %		58·3	54·5	60·4	47·4	56·0	54·9	46·6	43·3	49·1			
Ca No.		4	3	3	2	4	3	3	2	3			

NOTE: L = calcium diet initially on left
R = calcium diet initially on right

Table 22A (Contd.)

Controls (contd.)

Bird No.	Day 0	1	2	3	4	5	6	7	8	9	Tot.	Ca %	Ca No.
207		34	54	68	78	52	74	64	82	57	563	57·7	7
L	77	51	56	50	37	46	44	54	25	49	412		
204		33	67	42	47	36	45	49	28	36	383	46·1	2
L	70	62	54	49	53	47	46	30	56	51	448		
C1517		32	104	27	31	25	3	1	2	0	225	35·7	1
L	141·	104	66	53	79	28	29	17	28	2	406		
C1361		38	67	42	45	50	62	67	50	50	471	46·2	3
R	111	83	55	93	83	59	49	64	80	83	649		
C1393		62	58	47	12	3	57	7	50	23	319	41·0	4½
R	89	55	44	47	59	65	21	80	31	57	459		
C1359		3	68	70	127	70	110	58	172	110	788	52·1	4
R	144	110	118	114	92	105	33	88	1	63	724		
Totals													
Ca diet		202	418	296	340	236	351	246	384	276			
Def diet	632	465	393	406	403	350	222	333	221	305			
Ca %		30·3	51·5	42·2	45·8	40·3	61·3	42·5	63·4	47·5			
Ca No.		1	4	1½	2	1	4	3	3	2			

NOTE: L = calcium diet initially on left
R = calcium diet initially on right

Table 23A

Results of Experiment 10.2.

Selection of calcium after exclusive access for 2 days

Deprived — Pre-exposure to calcium diet

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	11	Tot.	Ca %	Ca No.
C2239	153	160	106	139	85	132	31	141	49	117	33	833	66·0	6
L			80	8	67	0	97	5	91	4	78	430		
C2244	89	92	43	51	46	49	48	76	47	64	32	456	48·6	2
L			61	53	64	54	53	43	50	42	62	482		
C2241	124	108	102	111	113	98	109	90	116	100	115	954	97·4	9
L			2	4	0	0	0	18	0	1	0	25		
C2250	112	121	74	89	72	91	80	89	36	79	30	640	64·0	7
R			62	47	73	40	61	14	23	6	34	360		
C2369	115	83	53	70	83	73	79	51	69	65	49	592	82·5	9
R			18	12	14	6	5	20	16	14	21	126		
Totals														
Ca diet	593	564	378	460	399	443	347	447	317	425	259			
Def diet			223	124	218	100	216	100	180	67	195			
Ca %			62·9	78·8	64·7	81·6	61·6	81·7	63·8	86·4	57·0			
Ca No.			4	4	3	4	3	5	3	5	2			

Pre-exposure to deficient diet

C2233			98	98	107	93	113	93	106	77	86	871	93·0	9
L	104	97	12	4	0	3	4	7	2	25	9	66		
C2376			34	72	61	71	104	91	111	116	118	778	80·9	8
L	111	84	64	29	47	17	16	3	3	0	5	184		
C2364			96	70	114	79	110	94	103	94	101	861	92·0	9
L	89	90	13	33	6	10	3	3	7	0	0	75		

Note: L = calcium diet initially on left
R = calcium diet initially on right

[Contd.

Table 23A (Contd.)

Deprived (contd.) Bird No.	Day 1	2	3	4	5	6	7	8	9	10	11	Tot.	Ca %	Ca No.
Pre-exposure to deficient diet														
C2247			32	110	54	116	88	125	96	142	109	872	61·6	7
R	117	129	129	41	97	32	66	40	50	29	60	544		
C2365			121	119	111	101	108	94	92	101	96	943	90·4	9
R	129	121	7	16	24	13	12	10	11	3	4	100		
Totals														
Ca diet			381	469	447	460	523	497	508	530	510			
Def diet	550	521	225	123	174	75	101	63	73	57	78			
Ca %			62·9	79·2	72·0	86·0	83·8	88·8	87·4	90·3	86·7			
Ca No.			3	5	4	5	5	5	5	5	5			
Controls					**Pre-exposure to calcium diet**									
C2245	125	151	94	62	73	32	78	64	121	60	92	676	48·9	4
L			82	102	79	115	71	68	29	103	57	706		
C2238	120	155	122	88	139	71	110	80	120	88	101	919	53·0	5½
L			100	92	57	101	103	106	78	88	90	815		
C2370	204	225	124	103	164	78	102	52	116	109	108	956	44·0	1
L			145	150	72	142	136	151	140	134	145	1215		
C2368	0	134	92	159	81	159	42	66	31	122	18	770	49·2	4
R			71	0	109	0	166	97	162	65	124	794		

[Contd.

NOTE: L = calcium diet initially on left
R = calcium diet initially on right

Table 23A (contd.)

Controls (contd.) — Pre-exposure to calcium diet

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	11	Tot.	Ca %	Ca No.
C2240	103	167	88	52	3	97	52	146	122	71	30	661	52·7	5
R			49	91	144	28	115	2	3	49	113	594		
Totals														
Ca diet	552	832	520	464	460	437	384	408	510	450	349			
Def diet			447	435	461	386	591	424	412	439	529			
Ca %			53·8	51·6	49·9	53·1	39·4	49·0	55·3	50·6	39·7			
Ca No.			4	1	2	2	2	1	3	2½	2			
Pre-exposure to deficient diet														
C2237			1	113	41	114	62	71	76	77	59	614	58·2	7
L	70	72	102	0	92	6	61	30	45	50	55	441		
C2375			45	89	48	97	98	54	31	116	42	620	35·4	1
L	79	102	99	107	144	131	142	114	138	92	164	1131		
C2371			1	124	95	131	121	159	88	149	77	945	71·6	7
L	90	89	117	2	59	0	41	4	69	3	79	374		
C2234			1	121	114	89	113	62	106	87	79	772	52·3	6
R	122	151	151	59	63	60	88	69	53	96	65	704		
C2251			8	79	67	75	79	100	60	110	66	644	59·9	7
R	84	110	102	32	54	32	45	28	61	13	64	431		
Totals														
Ca diet			56	526	365	506	473	446	361	539	323			
Def diet	445	524	571	200	412	229	377	245	366	254	427			
Ca %			8·9	72·5	47·0	68·8	55·6	64·5	49·7	68·0	43·1			
Ca No.			0	4	3	4	4	3	3	4	3			

NOTE: R = calcium diet initially on right
L= calcium diet initially on left

Table 24A

Results of Experiment 10.3.

Selection of calcium after exclusive access for 4 days

Deprived		Pre-exposure to calcium diet											
Bird No.		Day 1	2	3	4	5	6	7	8	9	Tot.	Ca %	Ca No.
P1426	Ca	4	40	117	115	154	66	161	115	155	651	93·9	5
						8	30	1	1	2	42		
D357	Ca	4	87	77	89	60	50	74	68	71	323	61·3	4
						67	41	33	20	43	204		
D421	Ca	63	84	94	98	74	59	59	67	60	319	58·3	5
						55	27	54	36	56	228		
D363	Ca	118	100	111	113	98	76	86	69	71	400	61·9	5
						67	15	64	37	63	246		
D419	Ca	83	144	146	166	116	118	134	111	115	594	73·2	5
						77	4	56	22	58	217		
Totals													
Ca diet		272	455	545	581	502	369	514	430	472			
Def diet						274	117	208	116	222			
Ca %						64·7	75·9	71·2	78·7	68·0			
Ca No.						4	5	5	5	5			
		Pre-exposure to deficient diet											
D359	Ca					109	52	83	76	75	395	56·3	4½
		102	95	94	95	71	27	76	58	75	307		
D416	Ca					103	61	116	133	117	530	70·1	3
		73	110	102	125	109	82	1	4	30	226		
D367	Ca					35	89	76	81	95	376	60·6	4
		84	100	101	111	108	17	56	23	40	244		
D356	Ca					55	58	164	72	140	489	61·7	3
		75	92	121	132	133	75	14	64	17	303		
D362	Ca					37	38	45	20	50	190	43·6	1½
		71	69	86	59	68	39	31	58	50	246		
Totals													
Ca diet						339	298	484	382	477			
Def diet		405	466	504	522	489	240	178	207	212			
Ca %						40·9	55·4	73·1	64·9	69·2			
Ca No.						1	2	5	4	4			

Table 24A (contd.)

Controls		Pre-exposure to calcium diet											
Bird No.		Day 1	2	3	4	5	6	7	8	9	Tot.	Ca %	Ca No.
D358	Ca	3	75	87	119	108	45	56	91	77	377	52·4	3
						60	65	118	36	64	343		
D414	Ca	4	2	0	0	5	25	50	66	37	183	25·2	0
						123	93	111	81	134	542		
P1416	Ca	82	88	83	96	63	71	52	77	66	329	48·5	2
						93	33	95	56	77	354		
D348	Ca	2	59	84	144	103	60	100	73	70	406	46·6	1
						79	92	107	92	95	465		
P1402	Ca	69	84	83	90	35	58	63	71	61	288	44·0	2
						114	30	89	54	79	366		
Totals													
Ca diet		160	308	337	449	314	259	321	378	311			
Def diet						469	313	520	319	449			
Ca %						40·1	45·3	43·3	54·2	40·9			
Ca No.						2	2	0	3	1			
		Pre-exposure to deficient diet											
D354	Ca					144	68	144	68	108	532	57·6	4
		57	66	96	149	110	51	63	98	70	392		
D353	Ca					36	2	58	31	39	166	30·1	0
		2	2	0	0	88	66	88	70	75	387		
P1425	Ca					78	77	70	66	53	344	52·5	2
		42	61	81	80	87	36	76	42	70	311		
P351	Ca					99	66	103	53	109	430	70·8	5
		67	82	83	104	32	30	14	46	55	177		
P1446	Ca					121	74	138	88	57	478	51·7	4
		104	119	133	166	118	69	43	80	136	446		
Totals													
Ca diet						478	287	513	306	366			
Def diet		272	330	393	499	435	252	284	336	406			
Ca %						52·4	53·2	64·4	47·7	47·4			
Ca No.						3	4	3	3	2			

Table 25A

Results of Experiment 11.1

Selection of calcium after an injection

Calcium Deprived - Gluconate Injection

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
C4742	2	32	26	30	63	37	80	31	49	40	390	56·9	6
	26	31	35	33	27	22	14	55	17	35	295		
C4753	0	3	1	1	106	1	100	11	101	0	324	36·5	3
	0	79	38	107	23	101	0	116	7	93	564		
C5089	18	11	11	50	30	23	32	66	73	60	374	63·7	4
	6	39	29	7	33	26	35	20	4	14	213		
C4749	26	17	76	1	95	60	67	117	72	112	643	57·6	7
	50	36	4	80	86	49	62	45	33	28	473		
C5503	1	16	4	51	87	106	82	60	65	78	550	71·7	7
	5	19	77	22	7	5	15	30	33	4	217		
C5500	0	0	0	2	24	14	32	16	30	16	134	31·4	2
	40	18	39	45	33	24	6	37	14	37	293		
C5496	4	16	65	26	48	37	39	45	42	49	371	44·0	2
	44	40	24	31	54	73	60	57	51	38	472		
C5949	22	17	25	55	73	75	72	73	90	63	565	66·9	6½
	4	62	30	55	33	20	12	24	8	31	279		
C5961	1	3	15	60	21	46	58	71	77	74	426	48·4	5
	26	45	47	53	72	55	50	44	36	26	454		
C5965	4	31	13	7	14	1	3	7	2	4	86	11·7	1
	2	16	52	119	42	102	91	75	91	58	648		
C6410	8	1	24	38	70	9	83	24	85	23	365	51·9	4
	26	42	39	34	22	76	16	38	9	36	338		
C6411	0	0	20	2	44	29	79	94	107	116	491	63·1	5
	0	21	45	78	36	66	7	19	6	9	287		
C6413	121	31	53	38	98	98	120	95	108	82	844	72·6	8
	20	26	48	43	31	17	21	48	27	38	319		
C6414	1	1	45	25	65	41	62	38	80	35	393	46·8	4
	12	61	31	51	31	65	17	82	23	73	446		
C6420	0	0	0	3	0	0	0	55	58	59	175	53·0	4½
	0	0	0	9	0	13	65	14	31	23	155		
C6432	0	0	7	31	31	30	17	43	26	28	213	37·8	1½
	0	0	28	39	43	71	44	37	45	43	350		
Totals													
Ca diet	221	179	385	420	869	607	926	791	1007	780			
Def diet	285	535	566	806	573	772	450	727	404	663			
Ca %	43·7	25·1	40·4	34·0	60·3	43·6	64·3	53·3	71·0	55·0			
Ca No.	6	4	4½	4½	9½	5	11	9	13	10			

Table 25A (contd.)

Calcium Deprived-Water Injection

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
C4741	85	1	86	1	100	44	93	47	64	50	571	77·1	7
	0	30	0	34	7	17	1	31	6	44	170		
C4750	47	5	28	17	69	64	82	113	84	91	600	75·9	7
	41	34	14	33	23	15	12	6	1	11	190		
C5085	82	54	57	94	130	109	138	137	116	124	1041	86·1	9
	4	22	22	30	16	21	15	15	4	19	168		
C5498	0	0	0	0	0	0	0	0	0	0	0	0·0	0
	68	48	71	64	79	57	63	70	45	64	629		
C5499	24	32	45	59	71	110	67	108	93	73	682	74·2	8
	60	39	34	15	25	14	35	6	6	3	237		
C5509	0	46	2	61	75	82	85	87	97	57	592	74·0	8
	75	6	49	1	0	22	1	15	20	19	208		
C5510	2	91	86	98	109	126	84	115	99	97	907	87·3	9
	80	1	16	1	1	10	13	1	9	0	132		
C5945	57	22	88	46	88	47	93	74	121	73	709	65·6	6
	50	41	9	98	1	76	14	50	7	25	371		
C5952	15	24	0	96	74	84	97	88	97	82	657	68·3	7
	81	46	92	27	14	14	1	16	8	6	305		
C5954	0	76	4	148	2	106	92	112	92	76	708	78·4	7
	68	3	58	0	62	1	0	0	0	3	195		
C5963	5	1	6	5	1	25	26	51	68	60	248	37·5	3
	3	50	52	92	69	56	52	35	20	22	451		
C6415	31	40	46	65	68	72	60	49	74	55	560	78·0	9
	27	16	26	0	8	5	18	5	18	35	158		
C6421	2	0	1	65	43	91	78	104	87	90	561	74·0	7
	2	56	70	16	42	0	3	2	1	1	193		
C6429	75	50	79	97	127	128	126	136	121	124	1063	90·1	9
	45	28	20	8	2	0	1	9	2	2	117		
C6435	5	1	0	24	79	76	83	56	70	53	447	46·3	5
	48	37	70	73	50	42	54	63	44	38	519		
C6439	25	40	67	60	65	50	83	82	92	67	631	61·3	8
	57	39	27	39	40	55	29	37	40	35	398		

Totals

Ca diet	455	483	595	1041	1101	1214	1287	1359	1376	1171
Def diet	709	496	630	487	424	427	306	354	256	308
Ca %	39·1	49·3	48·6	68·1	72·2	74·0	80·8	79·3	84·3	79·2
Ca No.	7½	7	9	10	13	12	14	14	15	15

Table 25A (contd.)

Normal Controls - Borogluconate Injection

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
C4752	8	80	64	91	70	79	80	101	65	75	713	46·1	4
	78	18	93	51	137	67	112	87	93	98	834		
C5088	11	80	3	86	82	42	89	103	48	83	627	51·9	6
	84	43	104	39	56	79	21	45	66	45	582		
C5091	0	14	16	21	15	40	5	60	3	89	263	31·2	1
	0	16	38	47	127	70	107	67	98	9	579		
C5502	0	11	9	72	36	119	21	73	21	42	404	45·7	4
	0	65	98	27	85	8	57	42	62	36	480		
C5504	0	46	51	35	37	78	82	94	77	93	593	43·2	3
	7	11	60	89	121	143	107	82	95	64	779		
C5508	0	13	75	46	79	106	91	88	93	76	667	47·2	6
	0	84	60	108	76	105	73	87	72	80	745		
C5511	20	9	22	38	44	50	78	45	120	48	474	37·2	2
	23	38	116	96	110	130	72	111	24	80	800		
C5944	0	0	0	66	94	96	109	17	1	5	388	72·5	5½
	0	0	0	64	2	32	6	36	2	5	147		
C5946	0	32	9	93	22	66	2	88	22	87	421	41·8	4
	0	88	79	57	85	44	105	26	88	13	585		
C5951	0	31	17	90	65	83	72	79	73	72	582	46·2	4
	0	65	77	86	58	64	68	83	103	73	677		
C5953	3	62	51	74	63	63	93	100	77	70	656	49·1	5½
	47	29	51	126	88	91	69	58	66	56	681		
C5958	4	33	28	167	54	26	123	4	137	17	593	46·0	4
	41	32	60	70	97	127	27	159	6	76	695		
C6409	0	0	59	23	75	55	62	60	81	43	458	53·9	4½
	0	0	0	53	32	60	49	73	39	86	392		
C6412	3	4	41	23	100	103	83	102	76	83	618	68·2	6½
	24	26	41	78	28	17	17	18	18	21	288		
C6416	0	3	33	84	75	131	119	102	88	36	671	51·4	4½
	0	3	99	28	83	76	84	108	73	80	634		
C6423	2	2	17	53	34	72	31	46	27	70	354	44·4	3
	2	10	46	16	49	37	75	79	87	43	444		
C6433	0	20	63	46	25	88	8	101	18	46	415	38·0	2
	0	76	71	94	99	51	104	21	109	51	676		
C6434	11	3	17	19	21	5	8	19	17	7	127	17·2	0
	45	45	64	38	25	73	51	89	76	104	610		
Totals													
Ca diet	62	443	575	1127	991	1302	1156	1282	1044	1042			
Def diet	351	649	1157	1167	1358	1274	1204	1271	1177	1020			
Ca %	15·0	40·6	33·2	49·1	42·2	50·5	49·0	50·2	47·0	50·5			
Ca No.	5	6½	3½	9	6	10	10	9	7	8½			

Table 25A (contd.)

Normal Controls - Water Injection

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
C4740	25	19	56	23	25	43	63	47	61	39	401	31·5	2
	107	75	54	96	125	96	78	92	60	86	869		
C4747	0	0	45	48	59	124	65	122	55	115	633	49·2	4½
	0	0	79	37	129	20	139	79	100	70	653		
C4751	111	60	109	48	99	52	81	77	73	60	770	57·9	5½
	48	60	1	69	67	73	62	73	62	71	569		
C5086	39	40	31	28	71	23	70	54	57	46	459	42·1	2
	73	71	57	75	73	89	48	64	29	52	631		
C5491	78	54	113	1	131	20	99	31	71	20	618	52·1	5
	16	39	2	118	2	142	19	106	43	81	568		
C5501	59	70	45	70	30	82	22	43	14	44	479	45·0	3
	32	1	54	17	87	65	83	72	94	60	565		
C5505	21	34	35	3	35	42	41	53	46	29	339	29·8	0
	86	54	90	92	60	93	87	83	82	72	799		
C5512	44	4	50	37	75	73	53	74	54	43	507	42·1	2
	83	86	76	79	49	74	66	52	71	62	698		
C5947	38	79	42	95	72	60	11	6	103	56	562	41·3	2
	82	5	94	107	96	95	58	110	66	87	800		
C5956	2	1	0	0	108	0	21	41	73	26	272	38·0	2½
	3	1	0	0	3	8	128	106	89	105	443		
C5962	0	0	0	1	0	0	0	0	0	2	3	0·3	0
	108	108	118	148	105	116	132	128	144	111	1218		
C5964	60	29	61	117	111	83	73	98	83	42	757	57·7	6
	23	46	49	75	35	66	76	45	67	71	553		
C6418	59	68	46	4	41	79	66	91	46	50	550	47·3	3
	17	58	72	86	80	46	67	48	72	68	614		
C6422	2	47	45	48	67	75	1	75	86	83	529	51·6	4½
	3	25	50	49	55	76	1	112	75	51	497		
C6424	77	68	63	68	87	78	97	70	107	81	796	51·1	5
	62	54	108	74	83	93	58	105	59	66	762		
C6425	61	70	73	82	87	72	111	88	93	77	814	54·8	7
	77	61	69	69	90	75	41	79	48	63	672		
C6430	36	24	44	44	44	44	44	62	44	57	443	39·3	1
	53	56	72	61	84	76	66	77	81	56	682		
C6437	48	11	91	1	76	2	78	26	107	3	443	46·2	4
	9	41	0	89	24	101	25	94	9	123	515		
Totals													
Ca diet	760	678	941	718	1218	952	996	1058	1173	873			
Def diet	882	831	1044	1341	1297	1404	1234	1523	1236	1355			
Ca %	46·3	44·9	47·4	34·9	48·4	40·4	44·7	41·0	48·7	39·2			
Ca No.	6½	8½	6½	4½	8	4	6½	6	10	5			

Table 26A

Results of Experiment 12

Selection of calcium when both choice diets are novel

In each case the calcium-supplemented diet is given first, and the deficient diet second.

PART I

Deprived

Bird No.	June 22	23	24	25	26	27	28	29	30	Jly 1	Tot.	Ca %	Ca No.
C3543	12	4	9	13	42	4	10	38	59	25	216	34·1	3
L	44	57	48	49	20	60	30	27	31	52	418		
C3535	76	89	122	114	127	123	132	124	86	78	1071	55·8	6
L	94	122	61	77	50	82	63	85	88	125	847		
C3551	0	63	49	123	147	157	165	191	142	149	1186	80·4	9
L	65	48	46	37	26	21	6	2	6	33	290		
C3542	11	34	9	28	9	57	19	54	93	99	413	81·6	7
L	40	13	15	1	16	2	2	3	0	1	93		
C3539	78	143	3	150	145	43	100	95	135	154	1046	77·0	8
R	50	20	36	9	24	26	31	43	48	25	312		
C3546	90	110	124	136	112	80	123	86	102	109	1072	78·0	9
R	33	35	14	10	22	37	31	32	43	45	302		
C3549	35	77	31	87	133	71	134	64	90	155	877	71·7	8
R	76	31	65	23	4	21	4	49	72	2	347		
Totals													
Ca diet	302	520	347	651	720	535	691	696	707	769			
Def diet	402	326	285	206	166	249	268	346	288	283			
Ca %	42·9	61·5	54·9	76·0	81·5	68·2	79·2	73·0	71·1	73·1			
Ca No.	2	5	3	6	6	6	6	7	6	5			

Controls

C3536	30	8	63	18	66	42	84	67	44	71	493	38·0	1½
L	39	80	46	110	69	111	84	94	86	86	805		
C3548	4	8	3	32	31	17	6	17	119	65	302	21·7	1
L	90	135	110	97	73	116	145	159	49	115	1089		
C3541	1	132	88	38	141	61	192	87	139	57	936	40·3	3
L	98	106	115	115	196	180	88	212	83	192	1385		
C3538	9	100	67	39	57	67	91	54	55	80	619	34·5	2
L	155	84	80	152	142	123	89	137	104	107	1173		

NOTE: R = calcium diet initially on right
L = calcium diet initially on left

Table 26A (contd.)

Controls (contd.)

Bird No.	June 22	23	24	25	26	27	28	29	30	Jly 1	Tot;	Ca %	Ca No.
C3540	55	3	144	83	115	107	90	152	145	107	1001	53·6	4
R	77	10	6	98	119	124	96	143	131	63	867		
C3537	2	0	1	1	49	0	1	0	3	1	58	4·3	0
R	74	0	92	164	168	165	175	147	154	140	1279		
C3550	83	108	173	157	85	69	95	70	68	78	986	53·9	3
R	111	89	25	36	90	93	118	87	94	99	842		
C3547	0	3	3	115	12	160	83	34	31	47	488	52·0	4½
R	0	3	3	0	44	51	141	95	88	25	450		

Totals

Ca diet	184	362	542	483	556	523	642	481	604	506
Def diet	644	507	477	772	901	963	936	1074	789	827
Ca %	22·2	41·7	53·2	38·5	38·2	35·2	40·7	30·9	43·4	38·0
Ca No.	½	4	3½	2	0	1	2½	1	3	2

Results - Part II

Deprived

Bird No.	June 26	27	28	29	30	Jly 1	2	3	4	5	Tot	Ca %	Ca No.
C3823	2	81	74	83	76	98	78	96	105	56	749	94·9	9
L	29	1	8	0	1	1	0	0	0	0	40		
C3827	0	1	1	37	28	60	64	93	110	81	475	93·0	7½
L	0	1	2	23	7	0	0	0	0	3	36		
C3835	0	92	86	87	100	106	130	106	115	101	923	65·0	9
L	101	60	67	68	54	44	7	66	27	3	497		
C3822	2	79	94	148	111	99	111	105	130	50	929	88·6	9
L	64	33	13	1	0	1	0	5	1	1	119		
C3828	110	140	117	143	109	121	120	130	113	90	1193	96·4	9
R	4	1	10	0	13	8	1	1	7	0	45		
C3836	64	19	137	147	149	136	173	147	153	111	1236	97·9	9
R	18	6	0	0	0	2	0	0	0	0	26		
C3821	14	8	38	42	57	34	65	84	84	46	472	50·4	5
R	33	50	60	49	28	73	30	48	57	36	464		

Totals

Ca diet	192	420	547	687	630	654	741	761	810	535
Def diet	249	152	160	141	103	129	38	120	92	43
Ca %	43·5	73·4	77·4	83·0	85·9	83·5	95·1	86·4	89·8	92·6
Ca No.	2½	5½	5	6	7	6	7	7	7	7

NOTE: L = calcium diet initially on left
R = calcium diet initially on right

Table 26A (contd.)

Controls — Results - Part II

Bird No.	June 26	27	28	29	30	Jly 1	2	3	4	5	Tot.	Ca %	Ca No.
C3830	39	113	58	83	46	2	22	29	50	22	464	43·2	2½
L	83	1	58	34	71	102	86	72	54	47	608		
C3831	10	59	63	52	23	24	27	12	6	1	277	22·2	0
L	53	81	77	86	110	121	101	122	143	77	971		
C3832	69	113	15	166	156	1	146	1	143	10	820	53·3	5
L	79	62	140	14	1	171	0	156	0	96	719		
C3829	41	38	90	69	42	61	30	87	6	94	558	43·3	3
L	20	97	60	96	99	89	102	49	116	3	731		
C3834	54	131	71	102	104	79	67	104	93	30	835	45·3	4
R	114	50	134	99	91	96	120	94	101	110	1009		
C3827	43	99	81	118	102	72	92	157	79	46	889	48·2	5
R	68	67	112	78	85	127	85	126	104	102	954		
C3825	21	128	84	64	9	156	118	116	129	88	891	44·6·	5
R	126	74	119	144	158	103	89	106	94	93	1106		
Totals													
Ca diet	277	681	462	654	481	395	502	506	506	291			
Def diet	543	432	700	551	615	809	583	725	612	528			
Ca %	33·8	61·2	39·8	54·3	43·9	32·8	46·3	41·1	45·3	35·5			
Ca No.	1	5	1½	4	3	1	3	4	2	1			

NOTE: L = calcium diet initially on left
R = calcium diet initially on right

Combined Totals of Part I and Part II

Deprived

Ca diet	494	940	894	1338	1350	1189	1432	1457	1517	1304
Def diet	651	478	445	347	269	378	306	466	380	326
Ca %	43·1	66·3	66·8	79·4	83·4	75·9	82·4	78·2	80·0	80·0

Controls

Ca diet	461	1043	1004	1137	1037	918	1144	987	1110	797
Def diet	1187	939	1177	1323	1516	1772	1519	1799	1401	1355
Ca %	28·0	52·6	46·0	46·2	40·6	34·1	43·0	35·4	44·2	37·0

Table 27A

Results of Experiment 13

Selection of calcium when familiar diet contains calcium and novel diet is deficient

Deprived

Bird No.	Jan 22	23	24	25	26	27	28	29	30	31	Tot.	Ca %	Ca No.
C8271	61	91	90	84	110	36	116	131	118	122	959	88·5	9
	0	3	28	5	20	10	15	6	20	18	125		
C8274	122	119	97	108	164	97	16	90	27	110	950	68·6	6½
	0	0	0	0	11	9	116	90	124	84	434		
C8276	67	85	119	93	175	114	156	104	131	116	1160	92·7	9
	1	0	0	0	2	24	0	39	5	21	92		
C8300	93	111	92	96	122	104	81	90	84	109	982	65·7	9
	86	25	58	26	74	33	46	72	47	55	522		
C8281	85	96	130	91	158	128	132	126	110	165	1221	98·4	9
	0	12	2	0	6	0	0	0	0	0	20		
C8269	33	41	39	24	89	31	68	68	54	75	522	66·7	7
	49	52	10	19	14	41	8	24	16	28	261		
C8287	109	98	79	38	91	64	48	33	55	78	693	50·2	2
	16	0	44	61	111	82	87	106	85	96	688		
C8289	1	56	88	81	130	111	129	143	117	137	993	91·5	9
	14	48	14	2	4	0	2	1	1	6	92		
Totals													
Ca diet	571	697	734	615	1039	685	746	785	696	912			
Def diet	166	140	156	113	242	199	274	338	298	308			
Ca %	77·5	83·3	82·5	84·5	81·1	77·5	73·1	69·9	70·0	74·8			
Ca No.	6	7	8	7	7	6	6	6½	7	7			
Controls													
C8285	47	75	86	70	140	105	117	76	85	41	842	72·6	8
	15	31	15	20	28	25	20	37	33	94	318		
C8278	18	59	56	93	103	101	104	133	90	135	900	49·5	6
	136	103	128	80	123	100	88	46	82	34	920		
C8293	97	102	130	72	115	127	155	150	80	168	1196	63·4	5½
	25	18	64	98	131	26	13	150	90	74	689		
C8264	0	106	115	111	133	119	114	116	116	133	1063	71·8	9
	122	86	63	14	45	0	24	26	14	24	418		

[Contd.

Table 27A (contd.)

Controls (contd.)

Bird No.	Jan 22	23	24	25	26	27	28	29	30	31	Tot.	Ca %	Ca No.
C8280	37	0	46	64	158	112	121	120	117	144	919	58·4	7
	88	115	105	34	84	68	56	35	67	2	654		
C8294	112	121	119	78	158	109	106	97	105	102	1107	98·0	9
	3	11	2	2	2	0	0	1	0	2	23		
C8273	111	81	163	127	175	80	188	121	148	167	1361	96·2	9
	0	47	2	1	4	0	0	0	0	0	54		
Totals													
Ca diet	422	544	715	615	982	761	905	813	741	890			
Def diet	389	411	379	249	417	219	201	295	286	230			
Ca %	52·0	57·0	65·4	71·2	70·2	77·7	81·8	73·4	72·2	79·5			
Ca No.	4	5	5	6	5	7	7	6½	6	6			

Table 29A

Results of Experiment 14.2

Selection of 10% alcohol (figures given are percentages)

		CONTROLS				Ca DEPRIVED			
	Date	P1403	P1410	P1408	P1421	P1401	P1411	P1444	P1450
Jan	17	61·8	86·5	72·7	4·0	87·2	40·8	45·2	50·0
	18	35·4	20·0	23·9	95·9	37·5	73·1	55·8	5·3
	19	41·9	39·1	55·2	30·4	82·0	45·4	67·4	46·9
	20	20·0	61·1	48·4	33·3	27·5	38·5	24·6	35·9
	21	5·2	5·3	14·3	3·8	15·4	3·4	5·4	7·4
	22	1·8	5·6	29·4	35·2	17·3	6·7	7·3	50·0
	23	1·5	6·9	60·0	11·1	11·1	4·3	11·8	16·1
	24	0·0	7·3	1·2	0·0	15·0	19·1	7·8	70·5
	25	0·0	5·1	39·1	7·1	8·1	3·4	17·6	0·0
	26	1·8	0·0	9·0	12·3	9·4	20·0	23·7	40·0
	27	3·4	29·5	13·4	3·4	3·8	3·8	13·3	2·4
	28	1·4	3·6	32·7	20·0	10·9	2·0	21·6	43·8
	29	0·0	4·4	1·6	2·0	8·7	1·6	12·1	4·6
	30	0·0	22·4	1·7	1·6	-	4·2	10·4	100·0
	31	0·0	2·2	1·1	5·1	5·7	1·2	9·3	0·0
Feb	1	3·0	14·3	1·4	3·3	7·8	2·0	4·0	21·4
	2	2·3	1·2	1·3	1·3	30·2	2·9	3·3	1·7
	3	2·7	41·4	1·8	5·7	16·4	1·8	3·2	11·1

[Contd.

Table 29A (contd.)

	Date	CONTROLS P1403	P1410	P1408	P1421	Ca DEPRIVED P1401	P1411	P1444	P1450
Feb	4	3·0	5·1	1·2	1·3	12·3	1·5	6·1	2·0
	5	1·3	34·3	1·1	2·1	24·6	2·7	9·9	3·8
	6	3·6	4·2	0·0	1·7	13·1	3·6	6·8	2·1
	7	5·8	30·5	1·2	3·6	50·9	2·6	5·1	6·0
	8	1·8	3·1	0·0	0·0	27·9	2·6	8·8	4·2
	9	1·7	37·0	0·9	6·9	45·6	6·7	8·5	1·6
	10	-	4·1	2·2	1·3	27·1	8·2	2·8	5·5
	11	0·9	71·0	0·0	2·9	55·6	71·8	6·2	0·0
	12	0·0	31·1	1·1	3·9	48·8·	54·5	4·9	1·5
	13	3·3	1·1	2·2	3·2	26·3	7·1	2·4	5·1
	14	4·7	25·3	1·1	1·2	85·5	43·4	29·4	2·0
	15	6·6	6·6	6·5	2·4	25·3	21·7	19·3	3·1
	16	2·2	59·2	0·0	1·2	56·4	60·2	34·9	8·1
	17	0·0	0·0	0·0	4·4	26·5	22·7	20·3	4·5
	18	11·5	70·8	2·7	0·0	14·2	51·9	21·5	1·2
	19	32·4	1·1	1·9	3·4	54·4	26·8	10·3	1·7
	20	5·4	79·2	0·9	1·8	27·0	52·9	33·8	5·1
	21	54·3	2·9	1·1	0·0	23·9	21·3	8·5	0·0
	22	30·7	95·2	3·2	1·6	31·0	54·4	33·8	5·5
	23	14·4	2·7	0·0	0·9	39·1	57·3	2·6	0·0
	24	10·1	62·3	7·6	2·5	15·8	21·7	40·7	3·6
	25	27·5	1·8	12·1	1·9	55·1	35·9	11·1	3·1
	26	6·7	62·8	5·4	5·4	72·8	62·1	33·3	1·6
	27	10·5	2·0	2·4	2·2	2·1	27·5	22·4	8·6
	28	7·7	87·1	3·6	1·5	54·9	53·1	45·9	8·6
Mar	1	25·7	2·7	0·0	1·8	6·3	21·3	16·0	3·3
	2	25·0	67·2	1·5	1·0	49·3	38·1	71·7	0·0
	3	16·5	2·6	1·7	1·0	19·1	29·1	12·4	1·8
	4	12·1	94·5	2·9	2·8	46·5	84·5	55·9	13·9
	5	6·0	1·7	1·3	2·7	26·5	10·3	6·8	4·8
	6	9·9	94·3	2·9	2·1	68·3	38·3	25·3	8·0
	7	17·5	18·6	1·5	0·0	45·6	3·8	11·5	5·9
	8	15·7	78·1	0·0	3·3	31·0	41·2	56·8	0·0
	9	44·3	7·5	-	-	22·8	3·1	13·3	6·7
	10	26·1	95·5	8·9	1·9	51·8	17·8	39·7	1·5
	11	77·7	6·4	26·1	2·5	25·0	2·9	60·0	7·5
	18	78·4	4·4	2·9	2·2	61·5	18·2	5·7	3·4
	19	26·8	93·5	1·3	9·1	63·3	22·2	55·2	11·1
	20	34·3	3·2	18·0	1·0	13·4	9·6	13·6	3·1
	21	58·3	29·1	0·0	6·7	59·5	11·9	43·2	8·9
	22	11·8	9·5	6·8	4·1	58·8	31·3	0·0	25·0
	23	52·1	11·5	10·5	3·3	21·2	86·6	11·1	32·3
	24	30·0	20·8	10·3	5·2	7·3	5·7	5·9	12·5

Table 30A

Results of Experiment 15

Selection of calcium under the influence of alcohol

Calcium deprived — Water

Bird No.	1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
C6803	2	74	35	87	139	104	62	115	182	85	885	80·4	8
	58	20	39	21	14	18	27	13	1	5	216		
C6795	18	76	86	92	102	89	100	104	139	82	888	90·0	9
	48	4	2	1	9	6	6	5	17	1	99		
C6810	54	121	87	93	131	115	121	148	147	136	1153	90·5	9
	31	27	22	24	9	3	3	1	0	1	121		
C6802	2	100	2	86	80	72	101	68	111	42	664	71·4	8
	67	0	88	11	10	12	0	16	56	6	266		
C7592	13	4	26	27	38	21	21	21	10	28	209	44·7	3
	27	22	7	40	29	39	25	22	29	19	259		
C7234	29	4	6	1	34	1	51	40	61	57	284	56·3	5
	31	25	36	31	1	44	9	21	8	14	220		
C7244	78	84	71	97	99	108	86	130	90	108	951	89·9	9
	22	4	19	0	0	1	0	2	29	29	106		
C7237	1	0	0	78	70	124	132	141	95	91	732	64·4	7
	55	77	93	39	57	37	38	7	0	2	405		
C7572	66	3	79	31	79	83	96	68	106	96	707	88·0	8
	15	42	2	17	2	7	1	4	2	4	96		
Totals													
Ca diet	263	466	392	592	772	717	770	835	941	725			
Def diet	354	221	308	184	131	1671	109	91	142	81			
Ca %	42·6	67·8	56·0	76·3	85·5	81·1	87·6	90·2	86·9	90·0			

Alcohol

C6811	60	24	42	61	80	74	70	85	123	59	678	74·9	8
	38	43	34	32	17	2	21	7	31	2	227		
C6798	41	25	45	44	35	31	45	27	43	35	371	77·1	9
	12	3	18	6	18	12	18	14	3	6	110		
C6812	2	0	7	1	0	0	0	0	0	2	12	4·0	0
	20	27	8	18	16	30	36	40	74	21	289		
C6806	5	4	1	0	24	2	2	6	36	53	133	27·3	2
	36	39	48	51	21	30	31	31	56	11	354		

[Contd.

Table 30A (contd.)

Calcium Deprived — Alcohol (contd.)

Bird No.	1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
C6799	2	0	34	0	83	1	55	38	142	70	425	60·4	6
	29	35	8	81	11	60	19	27	6	3	279		
C7583	30	47	9	45	32	102	64	59	42	65	495	71·9	7
	74	0	16	33	49	3	2	3	9	4	193		
C7578	55	60	70	56	79	87	90	110	95	98	800	96·4	9
	15	7	2	3	2	1	0	0	0	0	30		
C7243	40	36	2	62	1	60	12	65	65	82	425	58·5	6
	74	17	66	4	70	2	65	1	0	2	301		
C7580	103	72	87	50	80	87	85	91	81	71	807	93·2	9
	14	3	1	4	1	6	0	5	3	22	59		
C7570	59	59	51	44	57	81	42	60	24	43	520	58·2	8
	80	50	46	13	34	25	24	10	54	38	374		
C7589	108	29	57	47	3	94	90	105	100	107	740	81·7	7
	3	55	16	6	55	6	3	7	6	9	166		
Totals													
Ca diet	397	327	405	410	474	619	555	646	751	685			
Def diet	392	279	262	251	294	177	219	145	242	118			
Ca %	50·3	54·0	60·7	62·0	61·7	77·8	71·7	81·7	75·6	85·3			
Ca No.	5	6	7½	8	7	8	8	9	8	10			

Controls — Water

C6817	8	1	8	38	89	13	96	60	101	28	442	34·7	2
	15	113	114	91	73	116	54	80	118	56	830		
C6807	2	1	2	0	0	1	0	0	1	1	8	1·0	0
	1	74	63	89	113	100	105	102	142	88	887		
C6804	1	0	45	0	44	1	0	1	69	2	163	16·5	1
	1	0	36	107	91	109	130	131	143	79	827		
C6801	45	72	30	93	48	80	25	81	83	74	631	53·1	5
	31	19	82	47	91	34	97	35	106	16	558		
C6797	5	74	6	59	18	43	18	69	54	59	405	46·7	5
	37	2	82	49	81	26	76	14	88	7	462		
C7588	65	16	46	10	77	60	80	62	84	96	596	51·1	4½
	54	74	71	77	46	73	47	62	33	33	570		
C7576	120	33	137	40	74	89	137	34	115	86	865	58·7	6
	10	95	32	91	35	86	28	139	34	59	609		

[Contd.

Table 30A (contd.)

Controls

Water (contd.)

Bird No.	1	2	3	4	5	6	7	8	9	10	Tot.	Ca %	Ca No.
C7574	23	68	58	83	72	108	74	99	79	105	769	48·0	4
	74	86	109	81	113	64	89	56	85	75	832		
C7585	71	0	27	30	41	64	23	100	4	96	456	34·5	3
	55	56	76	45	61	60	83	17	102	10	565		
C7235	40	10	68	1	75	20	68	45	69	49	445	46·4	4
	15	70	40	72	14	82	38	75	34	74	514		
C7242	38	58	58	64	55	72	78	67	14	56	560	46·4	5
	74	60	71	36	79	47	46	58	100	76	647		
Totals													
Ca diet	418	333	485	418	593	551	599	618	673	652			
Def diet	367	649	777	785	797	797	793	769	985	573			
Ca %	53·2	33·9	38·4	34·7	42·7	40·9	43·0	44·6	40·6	53·2			
Ca No.	6	2½	3	4	4	6	5	5½	3	6			
					Alcohol								
C6808	1	13	44	52	66	40	48	57	60	37	418	45·8	5
	1	108	51	57	39	35	42	44	90	28	495		
C6795	2	0	102	7	84	4	69	14	103	20	405	41·6	4
	67	100	2	87	30	87	31	81	45	38	568		
C6185	17	9	11	13	55	17	58	16	94	22	312	42·4	3
	86	60	41	48	7	64	4	62	15	36	423		
C6800	24	45	20	55	77	78	56	69	72	51	547	46·1	5
	80	70	89	66	74	47	50	42	91	32	641		
C7575	17	31	76	32	61	13	53	42	24	25	374	44·2	3½
	75	43	3	32	1	94	21	57	71	75	472		
C7582	14	11	47	36	9	3	0	22	72	20	234	38·9	3½
	79	70	27	0	47	15	0	25	22	82	367		
C7590	53	24	21	35	33	56	41	55	79	10	407	35·3	1
	81	87	91	44	88	86	56	59	33	42	344		
C7579	25	80	50	50	80	112	80	93	78	60	708	67·3	9
	86	23	34	24	27	19	48	8	33	42	344		
C7587	0	0	0	2	4	42	1	33	18	36	136	11·3	0
	79	73	134	112	112	114	136	108	107	94	1069		
Totals													
Ca diet	153	210	371	282	469	365	416	401	600	281			
Def diet	634	634	472	470	425	561	388	486	507	547			
Ca %	19·4	24·9	44·0	37·5	54·9	39·4	51·7	45·2	54·2	33·9			
Ca No.	½	1	4	2½	6	3	6½	3	5	3			

Table 31.1A

Results of Experiment 16

Selection of calcium under the influence of a tranquilliser

In each case the calcium-supplemented diet is given first, and the deficient diet second.

Part 1

Bird No.	Jun 29	30	Jul 1	2	3	4	5	6	7	8	Tot.	Ca %	Ca No.
2327	44	22	103	24	92	36	73	71	81	70	616	63·5	6
	36	42	13	58	15	56	38	40	27	29	354		
2336	21	42	40	17	37	55	57	62	56	42	429	54·8	6
	50	10	33	50	47	24	21	38	28	53	354		
2331	52	35	50	53	50	67	68	85	83	106	649	83·1	9
	20	10	10	23	22	24	17	4	2	0	132		
2788	51	35	55	64	66	59	82	65	67	72	616	91·3	9
	18	11	9	2	1	8	0	5	0	5	59		
2338	11	28	33	43	43	41	49	19	35	44	346	53·4	7
	17	14	15	25	22	24	31	73	40	41	302		
2424	58	30	65	55	64	53	56	35	46	57	519	47·1	3
	67	57	50	69	38	71	59	48	79	45	583		
1044	0	13	1	0	2	1	3	0	1	1	22	5·1	0
	27	23	39	46	34	43	36	53	53	52	406		
Totals													
Ca diet	237	205	347	256	354	312	388	337	369	392			
Def diet	235	167	169	273	179	250	202	261	229	225			
Ca %	50·2	55·1	67·2	48·4	66·4	55·5	65·8	56·4	61·7	63·5			
Ca No.	3	4	6	3	5	4	5	4	4	5			

Part 2

Bird No.	Nov. 11	12	13	14	15	16	17	18	19	20	Tot.	Ca %	Ca No.
6106	1	3	96	47	73	83	105	102	88	86	684	83·4	8
	28	50	56	1	0	0	0	2	0	0	136		
6059	63	79	77	101	108	70	118	111	117	87	931	82·1	9
	17	6	34	23	6	26	13	26	15	37	203		
5467	12	31	68	66	56	55	52	60	64	52	516	61·6	8
	33	55	36	23	28	31	42	31	18	25	322		
6093	47	47	59	36	61	79	79	81	70	90	649	58·6	8
	27	42	50	65	49	53	42	39	53	38	458		

[Contd.

Table 31.1A (contd)

Part 2 (contd.)

Bird No.	Nov. 11	12	13	14	15	16	17	18	19	20	Tot.	Ca %	Ca No.
6090	4	21	11	42	7	38	5	44	12	47	231	38·8	5
	7	11	42	21	55	26	74	32	64	33	365		
6083	25	34	41	37	56	67	73	77	85	78	573	64·2	7
	38	45	56	31	29	27	28	31	17	15	319		
Totals													
Ca diet	152	215	352	329	361	392	432	475	436	440			
Def diet	150	209	276	164	167	163	199	161	167	148			
Ca %	50·3	50·7	56·0	66·7	68·4	70·6	68·5	74·7	72·3	74·8			
Ca No.	2	3	4	5	5	6	5	6	5	6			

Intake of S.U. 9064

Part 1

Bird No.	Day 0	1	2	3	4	5	6	7	8	9	10	Average
2327	200	165	215	285	270	235	230	295	195	260	260	237 ml.
2336	130	155	120	140	175	225	190	220	160	175	175	170 ml
2331	105	135	40	90	130	125	135	160	140	110	150	120 ml
2788	120	110	80	105	95	115	100	110	110	100	110	105 ml
2338	245	180	115	240	170	220	120	210	225	170	160	187 ml
2424	280	325	180	255	300	380	205	330	285	215	215	270 ml
1044	85	105	90	110	160	10	260	170	145	150	160	130 ml

Part 2

6106	110	145	190	230	185	180	210	230	170	185	184 ml
6059	45	85	385	175	250	165	235	235	205	180	196 ml
5467	145	170	180	135	145	105	170	140	115	135	144 ml
6093	165	170	200	210	210	195	185	220	185	190	193 ml
6090	40	75	100	150	170	120	160	170	160	150	130 ml
6083	120	130	200	140	170	160	180	210	160	150	162 ml

Table 31.2A

Results of Experiment 17.2.

Selection of Suspension of 1% $CaCO_3$

Deprived Group

	D 99			D 92		
Day	Calcium	Water	Calcium %	Calcium	Water	Calcium %
1	210	230	47·7	150	165	47·6
2	35	445	7·9	115	265	30·3
3	210	195	51·9	155	285	35·2
4	70	385	15·4	385	265	59·2
5	230	240	48·9	310	175	63·9
6	80	195	29·1	420	265	61·3
7	335	240	58·3	235	290	44·8
8	215	200	51·8	180	95	65·5
9	265	135	66·3	50	360	12·2
10	265	255	51·0	180	115	61·0
	P1437			C8788		
1	290	125	69·9	250	125	66·7
2	280	135	67·5	165	210	56·0
3	160	70	69·6	175	200	46·7
4	260	65	80·0	185	275	40·2
5	175	100	63·6	140	270	34·1
6	170	85	66·7	150	210	41·7
7	250	165	60·2	95	220	30·2
8	240	150	61·5	145	250	36·7
9	250	55	82·0	135	215	38·6
10	265	60	81·5	260	310	45·6

Table 31.2A (contd.)

Control Group

Day	Calcium	Water	Calcium %	Calcium	Water	Calcium %
	C 8829				C 2931	
1	95	435	17·8	45	305	12·9
2	255	450	36·2	80	200	28·6
3	110	515	17·6	50	315	13·7
4	210	395	34·7	145	320	31·2
5	290	465	38·4	90	290	23·7
6	200	595	25·1	105	325	24·4
7	200	440	31·3	60	355	14·4
8	250	540	31·6	95	360	20·9
9	275	485	36·2	135	300	31·0
10	105	255	29·2	55	285	16·2
	C 8920				D 2173	
1	50	180	21·7	160	260	38·1
2	65	265	19·7	55	265	17·2
3	95	195	32·7	65	365	15·1
4	55	275	16·7	20	340	5·6
5	90	220	29·0	30	320	8·6
6	40	200	16·7	35	340	9·3
7	130	205	38·8	20	430	4·4
8	95	210	31·1	25	470	5·3
9	30	130	18·8	15	350	4·1
10	75	240	23·8	50	370	11·9

Table 32A

Results of Experiment 18.2

Selection of Sodium Chloride solution by sodium deprived chickens
(Figures given as ml)

Date	N.W.B.		D 3377		D 3374		D 3382		D 3376		D 3386	
	H_2O	NaCl	H_2O	NaCl	H_2O	NaCl	H_2O	NaCl	H_2O	NaCl	H_2O	NaCl
1	22	57	85	57	27	24	37	52	24	43	47	18
2	20	3	62	0	50	7	55	7	32	7	48	7
3	90	5	50	15	35	10	35	5	30	10	45	10
4	5	0	0	0	25	0	35	15	25	0	55	5
5	45	10	40	10	35	15	90	20	25	15	105	0
6	20	65	15	60	0	20	55	0	30	5	60	10
7	80	5	50	5	35	5	60	5	30	5	85	10
8	10	65	0	105	0	90	70	0	45	0	40	0
9	40	10	40	0	35	5	0	95	35	0	70	10
10	0	105	0	125	0	85	10	80	50	10	65	10
11	60	0	65	0	65	15	0	145	45	0	45	20
12	5	105	50	120	0	105	75	5	55	5	50	30
13	75	5	55	5	80	5	40	0	45	0	40	15
14	0	95	25	25	0	110	60	0	35	5	35	15
15	90	0	65	5	80	20	40	0	35	5	35	10
16	0	30	-	-	20	45	50	5	15	0	5	30
17	85	0	75	0	115	0	35	15	55	5	50	5

Percentage of fluid ingested as NaCl solution:

Days 1 - 4 as 1% NaCl

32·2 26·8 23·0 32·8 35·1 17·0

Days 5 - 17 as 0·5% NaCl

49·3 48·9 52·8 38·7 9·9 19·4

Table 33A

Results of Experiment 19

Selection of 0·5% Sodium Chloride in the food by sodium-deprived Chickens.

In each case the sodium-supplemented diet is given first and the deficient diet second.

Deprived Birds

Bird No.	June 18	19	20	21	22	23	24	25	26	27	28	29	Total	Na %
D2942	85	92	79	138	161	100	122	101	100	195	60	62	1295	64·8
	120	55	117	65	0	56	24	63	66	25	78	34	703	
D3094	2	45	9	56	53	18	33	54	43	60	61	20	454	67·5
	28	6	59	8	4	31	9	18	6	0	2	48	219	
D2939	49	27	61	46	6	36	17	60	30	75	71	65	543	49·3
	57	52	42	86	94	21	80	25	43	0	16	32	548	
D3100	65	16	27	3	2	72	129	117	112	93	123	55	814	56·3
	48	98	108	125	129	17	1	10	0	0	1	96	633	
D3119	38	54	51	56	20	33	8	46	10	39	6	44	405	56·3
	30	7	8	9	44	11	57	15	45	19	47	22	314	
D 2932	65	52	74	44	120	4	103	1	21	1	126	4	615	45·3
	40	96	19	89	2	105	0	133	1	169	4	84	742	
D3114	87	3	10	20	66	25	51	3	37	20	67	47	436	28·0
	79	101	124	126	84	80	64	105	109	96	74	81	1123	
Totals														
Na diet	391	289	311	365	428	288	463	382	353	483	514	297		
Def diet	402	415	477	508	357	321	235	369	270	309	222	397		
Na %	49·4	41·1	39·5	41·8	54·5	47·2	66·3	50·8	56·7	61·0	69·8	42·8		

Table 33A (contd.)

Control Birds

Bird No.	June 18	19	20	21	22	23	24	25	26	27	28	29	Total	Na %
D2937	80	63	81	63	95	83	48	45	48	49	37	79	771	45·0
	27	74	74	115	62	34	82	95	93	83	116	86	941	
D2940	5	91	78	75	105	66	102	105	50	71	47	95	890	55·4
	68	31	65	84	66	49	45	52	115	41	62	39	717	
D3124	27	78	22	94	74	76	68	87	72	165	91	86	940	52·5
	44	39	95	75	82	50	74	83	71	56	73	108	850	
D3102	75	71	34	91	116	89	118	11	163	115	120	121	1124	70·7
	56	56	89	36	1	4	0	105	0	1	0	118	466	
D3113	3	52	50	100	83	83	92	157	102	106	81	57	966	51·7
	74	55	91	68	106	67	78	23	78	82	111	70	903	
D3108	0	17	2	3	31	16	49	61	49	79	17	67	391	35·7
	67	61	87	97	77	60	40	35	42	20	81	38	705	
D3111	30	101	86	100	96	90	68	88	78	80	62	89	968	53·9
	57	42	87	80	63	51	82	73	70	81	70	73	829	
D3097	34	39	6	0	3	2	1	3	48	43	34	75	288	21·4
	62	66	111	113	122	91	103	111	60	76	78	65	1058	

Totals

Na diet	254	512	359	526	607	505	546	557	610	708	489	669	
Def diet	455	424	699	668	579	406	504	577	529	440	591	597	
Na %	35·8	54·7	33·9	44·1	51·0	55·4	52·0	49·1	53·6	61·7	45·3	52·8	

Table 34A

Results of Experiment 20

Weights of Sodium-Deprived and Control Chickens

Deprived		Control	
Bird No.	Weight (gm)	Bird No.	Weight (gm)
D5422	1195	D5459	1820
D5423	1367	D5472	1710
D5461	1116	D5424	1135
D5420	1255	D5436	1190
D5468	1362	D5469	1500
D5449	1565	D5460	1640
D5426	1612	D5443	1975
D5448	1090	D5456	1410
D5444	1260	D5471	1060
D5440	1490	D5470	1775
D5458	1425	D5466	1585
		D5455	2260
Average	1363 gm		1579 gm

Table 35A.

Results of Experiment 20

Selection of NaCl at 0·15% and at 1·5%

NOTE: Na diet coloured pink, contained 0·15% Na from Sep. 11-15, and 1·5% Na from Sep. 16-27.

Deprived

Bird No.	Sep. 11	12	13	14	15	16	17	18	19	20	21	22	Total	Na %	Na No.
D5422	42	83	33	67	94	106	47	41	15	41	9	20	598	56·5	6
	61	13	50	0	4	0	67	30	79	35	80	41	460		
D5423	78	33	41	14	51	37	30	13	25	1	3	1	327	37·0	1
	32	63	31	41	54	65	59	55	28	36	60	33	557		
D5461	16	23	7	0	34	1	0	1	0	0	1	1	84	8·8	0
	62	51	61	57	66	94	95	75	80	82	82	71	876		
D5420	100	46	30	21	16	26	3	0	2	19	1	6	270	26·7	1
	13	38	50	49	80	60	80	72	85	62	80	71	740		
D5468	75	54	26	7	20	3	40	6	15	5	16	10	277	39·2	3
	1	0	25	25	42	75	18	45	33	55	41	70	430		
D5449	86	51	62	28	63	50	29	21	7	2	1	2	402	78·5	10½
	32	0	7	0	0	34	15	11	6	0	3	2	110		
D5426	83	8	43	55	74	19	48	31	6	43	2	2	414	51·5	6
	45	19	37	0	36	36	42	22	18	4	68	69	396		
D5448	16	53	11	25	7	34	2	2	0	8	1	2	161	14·4	2
	69	44	94	23	102	84	98	75	91	94	95	85	954		
D5444	86	37	40	2	16	2	3	2	1	18	2	1	210	17·4	0
	0	55	52	76	79	122	94	94	110	76	117	119	994		
D5440	39	37	27	35	16	30	20	55	17	41	14	63	394	47·5	5
	41	27	49	3	67	51	50	5	39	14	61	29	436		
D5458	94	88	66	58	60	82	60	77	88	96	49	90	908	66·0	10
	32	32	63	19	70	50	38	12	11	18	45	78	468		
Totals															
Na diet	715	513	386	312	451	390	282	249	176	274	99	198			
Def diet	388	342	519	293	600	671	656	496	580	476	732	668			
Na %	64·8	60·0	42·7	51·6	42·9	36·7	30·0	33·4	23·3	36·5	11·9	22·9			
Na No.	7	7	5	6	3	3	4	5	2	4	1	2½			

Table 35A (Contd.)

Deprived (contd.)

Bird No.	Sep 23	24	25	26	27	28	29	Total	Na %	Na No.
D5422	21	64	2	39	29	19	26	200		
	50	10	51	6	29	19	21	186	48·2	3
D5423	4	26	2	45	4	61	4	146		
	55	37	57	2	48	5	60	264	64·4	5
D5461	1	0	1	0	0	0	2	4		
	64	47	58	53	72	48	64	406	99·0	7
D5420	7	46	10	55	43	52	35	248		
	73	27	71	32	56	30	52	341	58·0	4
D5468	34	6	80	5	83	9	20	237		
	28	68	3	77	5	73	71	325	57·8	4
D5449	116	9	69	1	113	0	133	441		
	35	91	1	125	4	53	3	312	41·4	3
D5426	36	10	32	1	30	0	42	151		
	84	19	67	56	80	17	63	386	71·9	7
D5448	20	51	7	96	19	62	15	270		
	75	41	100	21	95	20	82	434	61·6	4
D5444	48	2	74	35	94	62	109	424		
	67	93	66	92	64	43	59	484	53·3	3
D5440	30	77	30	65	10	44	14	270		
	25	2	17	1	48	2	57	152	36·0	2
D5448	35	49	32	82	64	35	59	356		
	64	57	92	41	73	83	78	488	57·8	6
Totals										
Na diet	620	492	583	506	574	393	610			
Def diet	352	340	339	424	489	344	459			
Na %	63·8	58·7	63·3	54·4	54·0	53·3	57·1			
Na No.	8	7	7	5	7½	5½	8			

NOTE: In this case the deficient diet is given first, and the sodium-supplemented diet second.

Table 35A (Contd.)

Controls

Bird No.	Sep. 11	12	13	14	15	16	17	18	19	20	21	22	Total	Na %	Na No.
D5459	0	93	51	70	66	101	68	86	85	92	100	73	885	66·4	11
	0	21	13	26	35	50	63	65	30	4	73	67	447		
D5472	37	21	19	1	1	19	0	2	0	0	1	3	104	12·0	0
	51	55	43	43	72	77	70	61	76	74	69	69	760		
D5424	11	1	5	1	2	23	0	2	1	16	6	5	73	11·9	0
	46	44	36	30	58	45	60	41	52	27	50	50	539		
D5436	0	0	7	0	1	1	0	1	1	0	0	2	13	1·7	0
	88	62	51	52	66	63	46	46	63	62	58	87	744		
D5469	40	53	30	51	38	79	57	14	66	79	71	68	646	55·0	7
	36	49	44	60	95	70	56	55	4	0	37	23	529		
D5460	24	3	21	2	28	8	25	2	7	6	22	6	154	22·6	½
	50	64	46	37	43	61	25	37	42	44	28	51	528		
D5443	128	122	73	35	82	111	21	24	6	0	58	2	662	56·5	6
	0	0	4	24	45	23	84	61	77	70	38	83	509		
D5456	3	22	8	27	25	57	15	26	18	24	25	29	279	58·5	8
	24	3	20	2	14	21	25	19	19	13	18	20	198		
D5471	0	4	8	24	16	48	17	31	2	61	5	54	270	43·7	4½
	42	48	35	24	24	16	51	17	50	0	37	4	348		
D5470	96	97	52	41	45	51	40	39	34	48	50	32	625	54·4	5
	20	6	46	31	69	55	62	37	58	39	53	48	524		
D5466	69	43	55	40	61	36	65	34	32	40	36	7	518	52·5	5½
	30	45	31	14	35	44	31	34	33	38	55	69	459		
D5455	74	41	85	27	70	41	65	11	69	30	45	14	572	53·1	4
	11	53	7	41	38	66	41	69	20	33	59	67	505		
Totals															
Na diet	484	500	414	319	435	575	373	272	321	396	419	295			
Def diet	398	450	376	384	594	547	614	542	524	404	575	638			
Na %	54·9	52·6	52·4	45·4	42·3	52·7	37·0	33·4	38·0	49·5	42·2	31·6			
Na No.	5	5	5	5½	5	5	5	5	3	6	4	4			

Table 35A (contd.)

Controls (contd.)

Bird No.	Sep 23	24	25	26	27	28	29	Total	Na %	Na No.
D5459	19	58	52	97	96	32	89	443		
	91	63	93	2	1	83	41	374	45·8	4
D5472	3	2	6	70	80	48	88	297		
	79	61	64	21	13	27	11	276	48·2	3
D5424	2	52	6	43	12	31	19	165		
	47	6	48	12	38	12	40	203	55·2	4
D5436	2	1	0	2	0	0	3	8		
	61	68	76	78	88	77	103	551	98·6	7
D5469	22	45	25	50	36	28	34	240		
	50	54	61	55	63	65	63	411	63·1	7
D5460	28	19	34	13	41	5	53	193		
	31	39	22	51	19	47	25	234	54·8	4
D5443	58	33	33	46	51	55	69	345		
	54	70	48	53	52	25	24	326	48·6	4
D5456	24	23	21	36	27	28	42	201		
	25	27	40	21	36	29	30	208	50·9	5
D5471	7	57	2	56	5	55	7	189		
	46	1	59	2	66	1	31	206	52·2	4
D5470	41	98	37	91	39	57	46	409		
	62	5	54	3	69	29	65	287	41·2	4
D5466	51	28	54	11	4	9	4	161		
	33	48	30	76	76	61	81	405	71·6	5
D5455	66	11	89	11	94	3	70	344		
	42	75	4	87	5	72	36	321	48·3	3
Totals										
Na diet	621	517	599	461	526	528	550			
Def diet	323	427	359	526	485	351	524			
Na %	65·8	54·8	62·5	46·7	52·0	60·1	51·2			

NOTE: In this case the deficient diet is given first, and the sodium-supplemented diet second.

Table 36A

Results of Experiment 21

Selection of 0·7% NaCl by Normal and Deprived Birds
(Figures given are inml)

	Deprived						Control			
Bird No.	D5437		D5451		D5446		D5467		D5457		D5478	
Day	Na	H_2O	Na	H_2O	Na	H_2O	Na	H_2O	Na	H_2O	Na	H_2O
1	190	180	5	250	0	360	30	195	5	275	125	155
2	5	140	15	220	10	270	140	80	100	210	45	130
3	10	125	0	315	260	125	10	170	5	220	25	150
4	5	175	0	175	5	170	180	15	5	175	30	180
5	5	205	0	240	20	175	15	165	10	160	30	195
6	5	220	0	330	25	250	90	100	10	190	50	290
7	5	140	5	295	10	120	30	175	15	80	0	95
8	75	125	215	0	440	0	180	115	45	65	95	115
9	45	120	55	0	20	200	25	135	20	115	40	95
10	80	125	0	105	155	70	220	95	10	70	140	90
11	25	205	0	70	80	165	380	75	10	145	205	110
12	25	115	0	10	5	155	10	170	10	130	100	85
13	20	185	10	135	115	125	200	95	5	145	155	165
14	65	95	5	85	15	165	10	215	5	90	5	205
15	65	160	175	15	65	190	115	90	10	245	150	140
16	20	165	100	15	15	210	5	195	5	110	10	225
17	40	105	55	60	15	160	105	90	5	175	150	115
Total	685	2585	640	2320	1255	2910	1745	2175	275	2600	1355	2540

Table 41A

Results of Experiment 24.2

Intake of water and 0·7% NaCl

	D8118		D8120		D8130		D8126		D8117		D8132	
Day	H_2O	Na	H_2O	Na	H_2O	Na	H_2O	Na	H_2O	Na	H_2O	Na
1	95	10	115	55	155	30	190	15	175	135	75	95
2	180	15	190	50	130	225	120	210	185	75	110	75
3	165	5	175	45	95	205	235	30	210	55	145	85
4	190	10	195	35	210	115	190	220	175	65	195	40
5	265	10	295	50	265	100	305	15	445	15	185	140
6	245	5	235	15	235	90	225	140	315	35	265	15
7	145	10	210	65	235	55	245	50	230	40	160	75
8	245	80	200	45	205	75	280	20	215	25	195	10
9	195	5	180	60	160	155	225	95	205	25	175	35

NOTE: Injection of 1·5% formalin given on Day 6.

Table 43A.

Results of Experiment 25.1

Selection of Thiamine-supplemented Diet

In each case the thiamine-supplemented diet is given first, and the deficient diet second.

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Th %	Th No.
Deficient													
D6775	0	1	25	11	29	119	1	118	0	113	417	39·9	3
	74	66	75	16	131	3	154	1	106	0	626		
D6758	0	40	6	39	56	76	72	51	48	48	436	44·0	4
	64	54	94	66	65	38	39	39	51	44	554		
D6774	27	76	54	82	73	70	22	83	20	83	590	61·5	7
	45	49	28	8	21	28	73	17	77	23	369		
D6776	0	116	62	115	38	94	41	85	59	64	674	65·8	7
	67	2	2	0	78	11	59	25	58	49	351		
D6765	0	0	0	124	82	71	40	57	53	59	486	47·2	4
	58	78	55	7	62	35	67	58	65	58	543		
Totals													
Th diet	27	233	147	371	278	430	176	394	180	367			
Def diet	308	249	254	97	357	115	392	140	357	175			
Th %	8·1	48·3	36·7	79·3	43·8	78·9	31·0	73·8	33·5	67·7			
Th No.	0	2	2	3	2	5	1	4	1	5			
Control													
D6772	64	95	65	91	79	68	72	45	49	64	692	56·1	6
	50	55	36	30	62	52	42	70	76	69	542		
D6780	23	23	26	82	24	78	105	105	63	72	601	63·9	6
	55	69	41	19	56	13	15	3	23	45	339		
D6754	78	56	38	66	72	58	76	67	68	77	656	88·4	9
	1	25	19	9	1	8	5	17	0	1	86		
D6642	119	62	42	76	67	66	78	73	40	62	685	57·6	6
	0	88	64	61	53	45	39	40	63	51	504		
D6673	59	12	31	19	68	31	61	58	20	60	419	45·3	4
	20	100	47	76	38	51	38	34	67	35	506		
Totals													
Th diet	343	248	202	334	310	301	392	348	240	335			
Def diet	126	337	207	195	210	169	139	164	229	201			
Th %	73·1	42·4	49·3	63·1	59·6	64·0	73·8	68·0	51·1	62·5			
Th No.	4	2	2	4	4	4	5	4	2	4			

Table 44A

Results of Experiment 25.2

Selection of Thiamine-supplemented diet

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Th %	Th No.
Deficient													
D7152	2	0	5	91	2	111	27	90	122	87	537	59·9	5
	4	64	70	2	88	1	92	1	0	38	360		
D7162	9	109	74	122	112	105	70	93	81	96	871	75·7	8½
	69	16	74	5	36	25	6	0	48	0	279		
D7139	1	0	104	116	123	111	106	100	116	105	882	85·6	8
	41	99	3	5	0	0	0	0	0	0	148		
D7161	27	35	34	56	69	70	70	69	40	86	556	69·1	8
	1	6	16	18	47	33	39	9	77	3	249		
Totals													
Th diet	41	144	217	385	306	397	273	352	359	374			
Def diet	116	185	163	30	171	59	137	10	125	41			
Th %	26·1	43·8	57·1	92·8	64·2	87·1	66·6	97·2	74·2	90·1			
Th No.	1	2	2½	4	3	4	3	4	4	4			
Controls													
D7142	2	0	2	3	66	39	65	59	67	70	373	40·3	5
	85	77	79	77	37	60	29	24	42	42	552		
D7156	3	0	0	0	0	48	64	64	57	10	246	35·8	4
	62	66	68	74	66	30	7	0	23	45	441		
D7141	3	2	0	0	0	56	13	75	52	66	267	37·9	4
	61	60	62	70	70	27	51	3	34	0	438		
D7149	3	2	3	0	0	0	0	0	2	0	10	1·1	0
	108	111	66	93	97	106	91	76	114	78	940		
D7147	1	0	81	4	33	24	35	14	64	26	282	24·6	1
	123	51	68	72	73	75	92	75	112	125	866		
D7144	5	30	64	37	51	62	51	46	73	63	482	50·3	6
	85	71	23	55	54	48	38	27	47	29	477		
Totals													
Th diet	17	34	150	44	150	229	228	258	315	235			
Def diet	524	436	366	441	397	346	308	205	372	319			
Th %	3·1	7·2	36·1	9·1	27·4	39·8	42·5	55·7	45·8	42·4			
Th No.	0	0	2	0	1	3	3	4	4	3			

Table 45A

Results of Experiment 25.3

Deficient

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Th %	Th No.
D8292	33	62	55	57	45	66	55	63	56	57	549	68·2	8½
	5	28	31	36	45	43	34	26	1	7	256		
D8294	2	79	75	77	48	66	53	59	60	45	564	86·1	9
	32	2	3	13	20	12	6	2	0	1	91		
D8300	2	109	101	100	68	81	80	81	54	78	754	92·3	9
	40	1	10	4	5	0	2	0	0	1	63		
D8291	2	93	115	125	107	128	57	70	77	62	836	93·4	9
	2	2	5	1	2	3	43	0	1	0	59		
D8289	3	3	116	120	74	85	49	73	60	42	625	71·3	7½
	20	111	11	6	20	18	19	0	4	42	251		
D8123	3	3	24	35	19	32	12	59	55	66	308	53·1	3
	10	56	47	40	40	41	35	1	1	1	272		
D8365	2	3	89	58	66	47	37	55	43	25	425	62·0	7
	28	80	4	35	10	30	17	6	12	38	260		
D8312	3	5	144	124	79	99	51	57	22	72	656	75·0	7
	48	88	2	6	1	2	0	1	68	3	219		
D8301	65	98	105	100	75	88	71	77	82	86	847	95·2	9
	2	13	11	8	4	1	2	1	1	0	43		

Totals

Th diet	115	455	824	796	581	692	465	594	509	533
Def diet	187	381	124	149	147	110	158	37	88	93
Th %	38·1	54·4	86·9	84·2	79·8	86·3	74·6	94·1	85·2	85·1
Th No.	2½	5	8	8	7½	8	8	9	8	7½

Table 45A (contd.)

Controls

Bird No.	Day 1	2	3	4	5	6	7	8	9	10	Tot.	Th %	Th No.
D8303	3	3	2	2	2	2	3	28	97	73	215	28·4	2
	50	32	33	104	80	99	73	62	2	6	541		
D8306	2	3	3	2	0	2	2	0	88	82	184	21·7	2
	59	59	54	127	87	104	69	81	18	7	665		
D8314	3	2	3	2	1	1	2	1	2	1	18	2·8	0
	65	61	67	131	85	93	37	34	28	35	636		
D8290	17	86	91	82	68	69	77	83	64	42	679	72·5	8
	57	7	19	20	14	33	15	11	24	57	257		
D8297	3	3	78	42	84	20	89	55	88	51	463	53·9	5
	68	52	25	76	2	88	4	21	2	58	396		
D8299	6	4	71	44	64	80	39	29	54	66	457	46·4	5
	86	82	51	64	26	32	44	61	37	44	527		
D8293	53	69	62	62	42	47	26	31	48	38	478	50·5	5
	61	46	56	50	44	34	35	52	34	57	469		
D8287	5	49	38	61	29	54	55	72	55	61	479	49·7	5
	80	56	83	65	65	42	24	10	25	35	485		
D8295	5	3	2	23	86	53	61	125	40	23	421	46·5	4
	85	58	33	87	26	39	44	0	79	81	532		
D8305	2	6	2	45	82	38	41	78	72	61	427	57·2	5
	30	32	78	73	24	49	4	0	1	29	320		

Totals

Th diet	99	228	352	365	458	366	395	502	608	498
Def diet	641	485	499	797	453	613	349	332	250	409
Th %	13·4	32·0	41·4	31·4	50·3	37·4	53·1	60·2	70·8	54·9
Th No.	0	2	4	2	5	5	5	5	8	5